

# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

Stomach Worm ( <i>Haemonchus contortus</i> ) Infection in Lambs and Its Relation to Gastric Hemorrhage and General Pathology (Key No. A-211)	1
JOHN S. ANDREWS	
Arrangement of the Tissues by Which the Cow's Udder Is Suspended (Key No. I-29)	19
W. W. SWETT, P. C. UNDERWOOD, C. A. MATTHEWS AND R. R. GRAVES	
A Micrurgical Study of Crown Gall Infection in Tomato (Key No. N. Y. (Cornell)-51)	45
E. M. HILDEBRAND	



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# JOURNAL OF AGRICULTURAL RESEARCH

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No. 1

## STOMACH WORM (HAEMONCHUS CONTORTUS) INFECTION IN LAMBS AND ITS RELATION TO GASTRIC HEMORRHAGE AND GENERAL PATHOLOGY<sup>1</sup>

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### INTRODUCTION

The life history of the stomach worm, *Haemonchus contortus*, was first worked out experimentally by Ransom (?)<sup>2</sup> in 1906. Nine years later Veglia (8) made a thorough study of this nematode, including observations on the pathological changes produced by it in the abomasum of experimentally infected lambs. He found that the worms caused considerable hemorrhage into the stomach during the fourth and fifth stages of their life cycle.

In 1931 Fourie (4) made the first detailed study of the pathology and hematology of *H. contortus* infection in sheep. He concluded that the anemia observed in sheep experimentally infected with this nematode was purely hemorrhagic in character, since he was able to reproduce the same blood picture in healthy lambs by periodic bleeding from the jugular vein. Three years later, Martin and Clunies Ross (6) made a "minimal" estimate of the quantity of blood withdrawn daily by *H. contortus* females. These authors calculated the quantity of phosphorus in the eggs laid by the worms, and, on the assumption that this phosphorus came wholly from the blood of the host, they estimated that 2,000 female worms would withdraw about 30 cc. of blood daily. They concluded that the total loss of blood as a result of such an infestation could safely be doubled, since the 30 cc. did not include the blood consumed by the male worms, that which undoubtedly passed through the alimentary tract of the worms unchanged, and that which was lost by hemorrhage into the stomach. They agreed with Fourie that the effects of heavy infestations with *H. contortus* were due to the continual loss of blood, without any additional action of toxic secretions or products of the parasite's metabolism.

Boughton and Hardy (1) were able to observe the behavior of this parasite through fistulas made in the fourth stomach of infected sheep. They found that the parasites attached themselves to the stomach wall by a peculiar striking motion of the head and neck; that they remained

<sup>1</sup> Received for publication November 6, 1941.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 17.

attached for about 12 minutes and sucked blood, which could be followed through the intestine of the nematode almost to the anus; and that minute hemorrhages, which continued for a maximum of 7 minutes, occurred as soon as the worms detached themselves from the stomach wall.

It is evident from the literature cited that *H. contortus* infection in sheep may cause severe anemia in these animals, but except for the report of Boughton and Hardy there is no direct evidence that this anemia is the result of hemorrhage.

It is the purpose of the present paper to present additional evidence to the effect that (1) infection of lambs with the common sheep stomach worm, *H. contortus*, may result in severe and even fatal anemia; (2) this anemia is chiefly due to hemorrhage into the fourth stomach; (3) the quantity of blood lost by the hemorrhage may be estimated by determining the quantity of blood passed in the feces; (4) the anemia produced by stomach worm infection is similar to that caused by the periodic withdrawal of measured quantities of blood by bleeding from the jugular vein; and (5) the anemia is the result, at least in part, of blood loss due to continuous, seeping, capillary hemorrhage resulting from injury caused by the worms to the mucous membrane of the wall of the stomach.

#### MATERIALS AND METHODS

Nineteen lambs 2 to 8 months of age were used in these experiments, which were conducted in 1933 and from 1936 to 1938, inclusive. All the lambs were born and raised at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., and were Hampshire-Southdown crosses. They were kept in cages having concrete floors, which were cleaned daily. The lambs were fed an adequate ration consisting of alfalfa hay and a grain mixture. Salt and water were kept before them at all times. At the beginning of the experiments the lambs were free of all nematode parasites except *Strongyloides papillosus*, which apparently did not affect the results of the experiments, since this parasite was present to the same extent in both control and infected lambs.

For the purpose of infecting the experimental lambs, female specimens of *Haemonchus contortus* were obtained from freshly slaughtered sheep at an abattoir near Washington, D. C. The eggs from these worms were cultured in helminthologically sterile sheep feces and animal charcoal, and the infective larvae were administered per os to a worm-free lamb by means of a funnel and rubber tube. When this lamb began to pass eggs of *H. contortus*, the feces were cultured with animal charcoal, and the larvae obtained were administered to the experimental lambs in the doses shown in table 1.

Whenever possible the length of time between the first dose of infective larvae and the first appearance of blood and stomach worm eggs in the feces was ascertained. At frequent intervals blood samples were taken from the jugular vein of all lambs, and records were made of the total number of red and white cells per cubic millimeter of blood, the number of grams of hemoglobin per 100 cc. of blood, the percentage of packed red cells, by volume, in 1 cc. of whole blood (hereafter referred to as volume percentage of packed red cells), the percentage of the



TABLE 1.—Data on degree of infection of lambs with *Haemonchus contortus* and first appearance of blood and worm eggs in feces

Lamb No.	Approximate age at beginning of infection	Date of administration of larvae	Larvae per dose	Doses	Total larvae	Period between first dose of larvae and appearance of—		Date of post mortem examination	Period between first dose of larvae and post mortem examination	Adult worms or larvae recovered
						Blood in feces	Worm eggs in feces			
	Mos.	1933	Number	Number	Number	Days	Days	1933	Days	Percent
1	2.5	May 18..... June 27..... July 20.....	1,500 30,000 45,000	1 1 1	75,500	-----	27	Aug. 1	75	26.36
2	2.0	May 7.....	2,000	1	2,000	-----	18	Dec. 9...	216	-----
3	4.0	July 6-Aug. 30..... Sept. 1-25.....	1,000 5,000	56 25	181,000	-----	14	Oct. 30..	116	.38
4	4.0	July 8-Aug. 30..... Sept. 1.....	500 1,000	84 1	43,000	-----	18	do.....	114	.86
5	3.0	July 13.....	35,000	1	35,000	-----	18	Aug. 27..	45	4.47
6	6.0	Sept. 8-Oct. 7.....	5,000	31	155,000	-----	22	Oct. 30..	52	2.13
7	8.0	Dec. 23.....	45,000	1	45,000	-----	6	1937 Oct. 25..	306	.002
8	8.0	Jan. 6.....	13,000	1	13,000	-----	10	Jan. 27..	21	4.53
9	6.0	Aug. 26.....	8,800	1	8,800	-----	8	Dec. 10..	106	-----
10	6.0	Sept. 17-18.....	12,500	2	5,000	-----	6	Sept. 25..	8	10.00
11	3.0	July 30.....	100,000	1	100,000	-----	10	1938 Aug. 22..	23	4.77
12	3.0	July 30-Aug. 23.....	5,000	25	125,000	-----	10	Aug. 24..	25	4.00
13	4.0	Aug. 27.....	70,000	1	70,000	-----	-----	Aug. 31..	4	3.14
14	4.0	do.....	70,000	1	70,000	-----	6	Sept. 3..	7	2.85
15	4.0	do.....	70,000	1	70,000	-----	7	Sept. 7..	11	2.85
16	4.0	do.....	70,000	1	70,000	-----	-----	Sept. 19..	23	2.85

<sup>1</sup> Approximate.

different types of white cells, and the number of reticulocytes per 1,000 red cells. The number of worm eggs per gram of feces passed by the infected lambs was also ascertained at frequent intervals. Observations on the icteric index were made, but since there were no deviations from normal these data are omitted.

The presence of blood in the feces was determined by the benzidine test. A Levy-Hausser counting chamber was used for obtaining the total red and white cell counts. A clinical model Haden-Hausser hemoglobinometer was used for the hemoglobin determinations, and a Wintrobe hematocrit was used for ascertaining the volume percentage of packed red cells. The blood smears from which the differential white cell counts were made were stained with Wright's stain. For the reticulocyte counts, the blood was stained in vitro with brilliant cresyl blue. Approximately 500 white cells and 1,000 red cells were counted for the two determinations.

In order to ascertain the relationship between the different stages of development of the worms within the host and the pathological changes produced in the abomasum, four lambs (Nos. 13, 14, 15, and 16) were given equal numbers of infective larvae on the same day and were then killed at different intervals after infection.

The total quantity of blood passed with the feces of two lambs (Nos. 11 and 12) during the period that they harbored fatal infections

of *H. contortus* was ascertained by a modification of the method published by Van Eck (2) for determining the quantity of blood in human feces. One gram of sheep feces was boiled with three separate 20-cc. portions of glacial acetic acid. The acid from the three extractions was filtered off by suction through a piece of No. 1 Whatman filter paper placed over a Büchner funnel, and was collected in a large test tube. The filtrate was then thoroughly mixed, measured, and a small known fraction of the total, the size of which depended on the quantity of blood in the sample, was treated with a 1-percent solution of commercial benzidine in glacial acetic acid and 3 percent hydrogen peroxide. After dilution with 95 percent alcohol and 5 percent sodium hydroxide in distilled water, the resulting solution was placed in a colorimeter and the color compared with that of a similarly treated standard containing a known quantity of blood. As a control on this phase of the work, a known quantity of blood was removed daily, except Sundays, from the jugular vein of lamb 17, and blood counts made to determine the degree of anemia produced. Lambs 18 and 19 also were uninfected and used as controls in determining the changes in red cells and hemoglobin in the blood.

#### DATA OBTAINED

##### WORMS RECOVERED AND FIRST INDICATIONS OF BLOOD AND EGGS IN FECES

The data on the degree of infection of the experimental lambs with *Haemonchus contortus* and the first appearance of blood and worm eggs in the feces are given in table 1.

Lambs 1, 11, and 12 died of *H. contortus* infection. Lamb 5 was apparently in the last stages of the disease, as is indicated in figure 4, when it was killed for post mortem examination. The remainder of the lambs recovered from the effects of the experimental infections administered and were autopsied on the dates indicated.

In those lambs in which blood or worms eggs appeared in the feces, the former was observed, on the average, about 8 days after infection and the latter about 20 days after. In lambs 11 and 12 death occurred before worm eggs appeared in the feces, thus making a positive diagnosis of *H. contortus* infection impossible prior to post mortem examination. There was a delay of a few days in the appearance of worm eggs in the feces of lambs 1 and 6. In lamb 1 this delay was no doubt due to the very large infestation present, resulting in an overcrowded condition and a retardation of development of the worms. In lamb 6, the delay was probably due to the fact that the lamb was able to build up some resistance to the infection as it had received comparatively large daily doses of larvae for a considerable period.

The percentages of larvae that were able to establish themselves in the host animals varied. However, since the interval between infection and necropsy also varied, it was impossible to determine whether there was a significant difference between the number of worms establishing themselves in the lambs that received only a few doses and in those that received daily doses for a fairly long period of time.

##### RED CELLS, HEMOGLOBIN, AND BLOOD RECOVERED IN FECES

Figures 1 to 8 show, for both experimental and control lambs, the changes in the number of red cells per cubic millimeter of blood, the

number of grams of hemoglobin per 100 cc., the volume percentage of packed red cells, and the number of worm eggs per gram of feces. Figures 5, 6, and 7 also contain data on the quantity of blood lost by lambs 11, 12, and 17, respectively.

#### LAMBS RECOVERING FROM INFECTION

Data on the red-cell counts and hemoglobin were obtained on all the lambs that recovered from the infection, but the results from only three representative lambs (Nos. 4, 3, and 9) are presented in this paper.

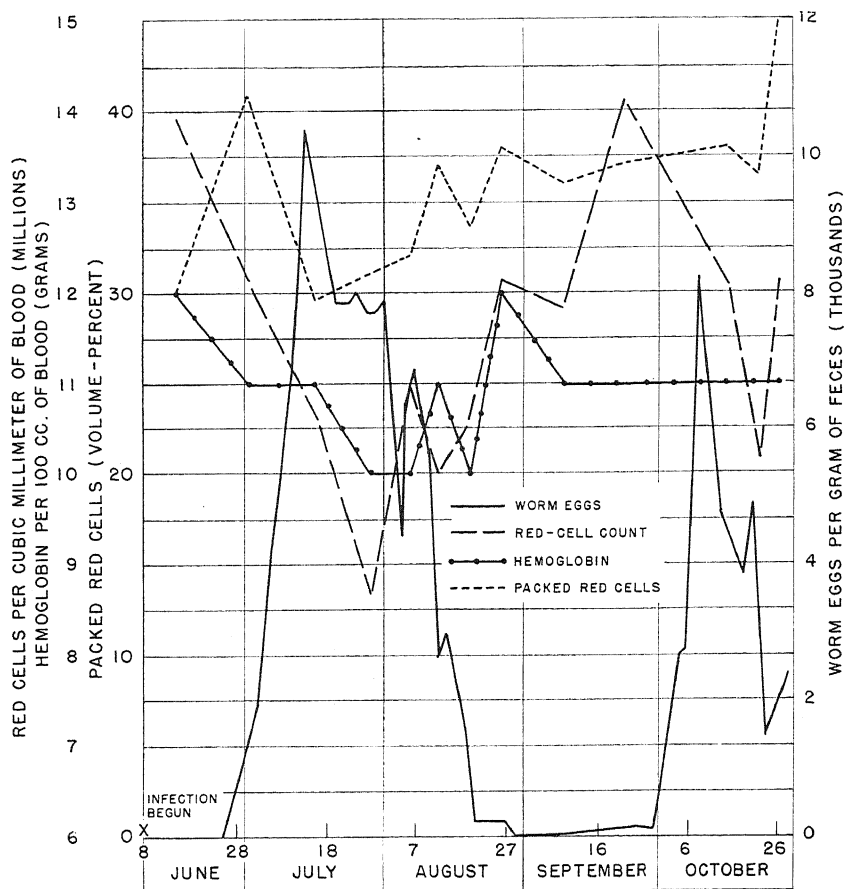


FIGURE 1.—Red cells and hemoglobin in the blood of lamb 4 correlated with the number of worm eggs per gram of feces passed during experimental infection with *Haemonchus contortus*.

The daily doses of larvae administered to lamb 4 (fig. 1) were apparently too small to produce marked symptoms of stomach worm disease, as is indicated by the relatively small decrease in the number of red cells per cubic millimeter of blood. Although the number decreased to 8.6 millions, it was not sufficient to cause marked changes in the hemoglobin or in the volume percentage of packed red cells.



The most significant observation recorded in figure 1 is the correlation between the low point of the red-cell curve and the high point of the worm-egg curve. Following the first peak in the egg count, the number of eggs decreased rapidly to almost zero, in spite of the daily administration of larvae. Simultaneously, there occurred a rapid increase in the number of red cells and in the hemoglobin. Approximately 5 weeks later there was a second increase in the number of worm eggs with a second slight decrease in the number of red cells, the two phenomena occurring again with approximately the same time relation between them. These data show a definite negative correlation between the number of worm eggs and the number of red cells in the blood. They also indicate that lamb 4 developed a partial resistance against superinfection, only to have it broken down temporarily a short time later by continued reinfection.

The greater number of larvae administered to lamb 3 caused a correspondingly greater decrease in the number of red cells, quantity of hemoglobin, and volume percentage of packed red cells (fig. 2) than in the case of lamb 4. In lamb 3 the number of red cells decreased to 6.5 millions per cubic millimeter of blood shortly after the number of eggs per gram of feces reached its maximum of 12,700. In this lamb also a sudden decrease occurred in the number of eggs per gram of feces in spite of continual superinfection, and there was also a simultaneous rapid increase in the number of red cells, quantity of hemoglobin, and volume percentage of packed red cells. As with lamb 4, a secondary increase in the number of eggs in the feces occurred simultaneously with a slight decrease in the number of red cells.

In lamb 9 (fig. 3), the changes brought about in the blood by the worm infection paralleled closely those in lambs 4 and 3. However, in lamb 9, the peak of the worm-egg curve reached 24,800 eggs per gram of feces, whereas the lowest point of the red-cell curve was 4.2 millions per cubic millimeter of blood. The low points of the curves for hemoglobin and for the volume percentage of packed red cells were reached about a month later than that for the number of red cells. Coincident with the decrease in egg count was the characteristic rapid recovery of the blood to normal within about 29 days. The peak of the egg-count curve of this lamb was much higher and broader than that of the two preceding lambs, owing probably to the fact that lamb 9 had no chance through superinfection to develop resistance against the infection.

In all three lambs that recovered from the infection, there was found to be a negative correlation between the egg count and (1) the number of red cells per cubic millimeter of blood and (2) the quantity of hemoglobin per 100 cc.

#### LAMBS DYING FROM INFECTION

In the study of the blood picture of the lambs that died of the infection, lamb 5 also was included since, as already stated, it apparently was in the last stages of the disease when it was killed for post mortem examination. Complete data on lamb 1 were not available; consequently, this lamb was omitted from the study.

The data for lamb 5 are given in figure 4. The blood picture of this animal differs from that of the foregoing ones in that there was no

tendency for the number of red cells and quantity of hemoglobin to return to normal with a decrease in the number of eggs per gram of feces. It also differs from the others in that the decrease in the number of red cells occurred before the peak of the egg count had been reached.

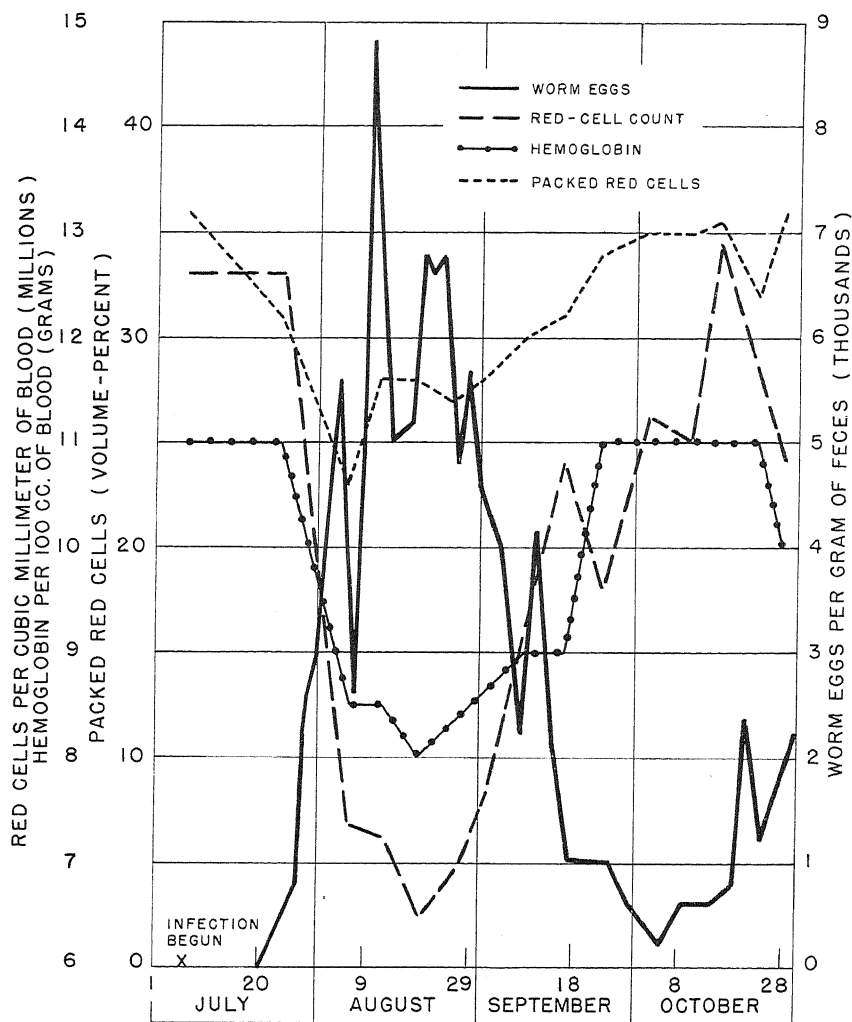


FIGURE 2.—Red cells and hemoglobin in the blood of lamb 3 correlated with the number of worm eggs per gram of feces passed during experimental infection with *Haemonchus contortus*.

In fact, most of this decrease occurred before the number of eggs per gram of feces rose to a figure that would be diagnostic of stomach worm disease. This lamb was anesthetized with ether and the stomach injected with hot Gilson's fluid in order to fix the worms in position in the stomach. When the worms were counted on post mortem exami-

nation, a very unusual sex ratio was discovered. There were 1,474 males and 92 females, a finding that accounts for the terminal drop in the egg count without any sign of an increase in the number of red blood cells.

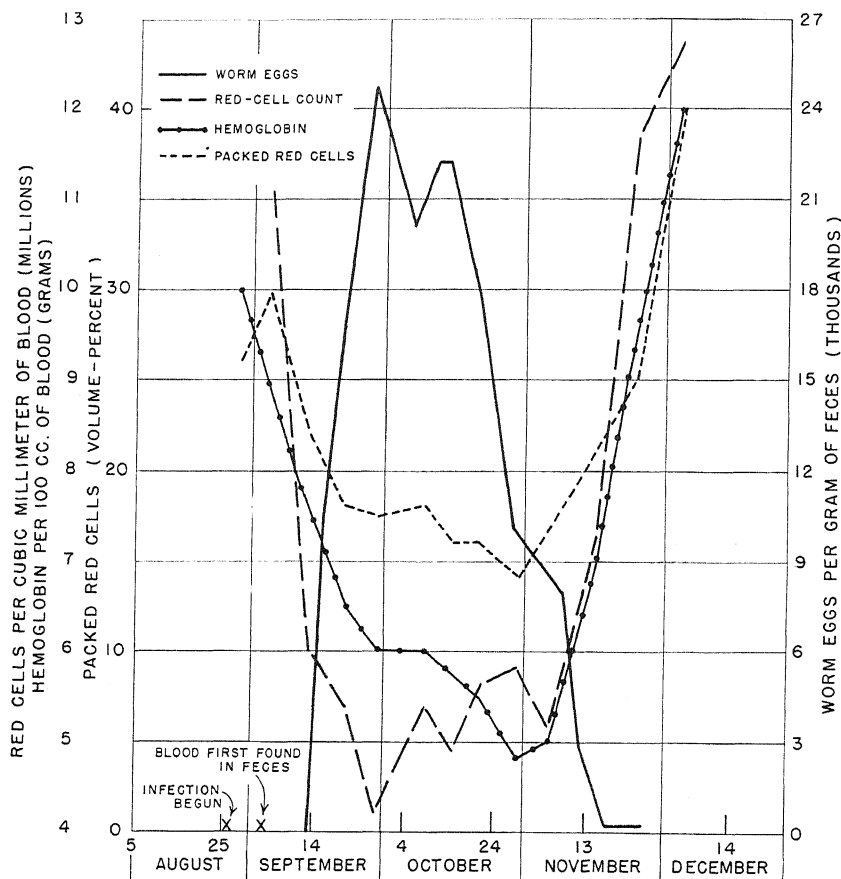


FIGURE 3.—Red cells and hemoglobin in the blood of lamb 9 correlated with the number of worm eggs per gram of feces passed after experimental infection with *Haemonchus contortus*.

Figures 5 and 6 present the results obtained for lambs 11 and 12, respectively. The blood pictures are similar to that of lamb 5 but, as already stated, no worm eggs were passed in the feces of either lamb 11 or 12. In both of the last-mentioned lambs, the number of red cells and the quantity of hemoglobin decreased to practically the same level at death.

According to figures 5 and 6, 1,492 and 2,380 cc. of blood were recovered from the feces of lambs 11 and 12, respectively, during the 10 days that the feces of these lambs were analyzed. Each of these lambs weighed about 25 pounds. According to Hodgson (5), who states that the weight in pounds multiplied by 38 gives the blood vol-



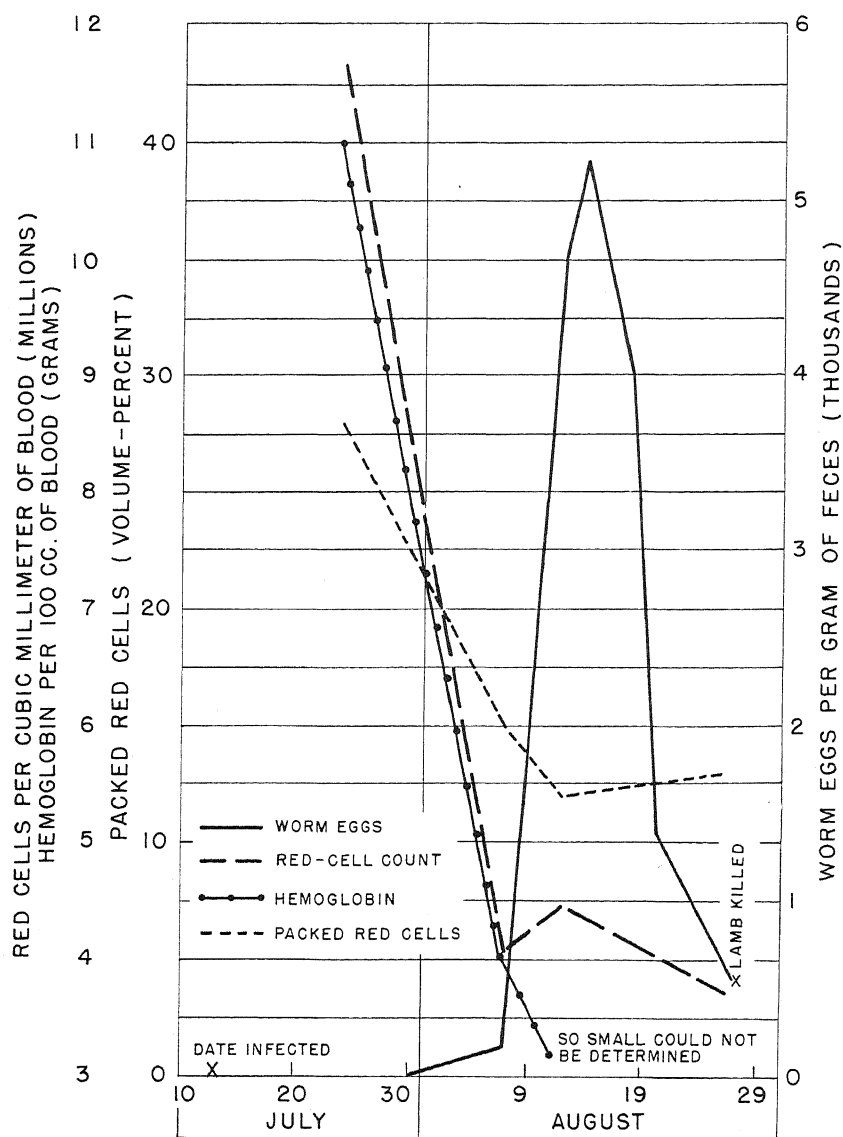


FIGURE 4.—Red cells and hemoglobin in the blood of lamb 5 correlated with the number of worm eggs per gram of feces passed during experimental infection with *Haemonchus contortus*.

ume, each animal normally had about 950 cc. of blood. Therefore, 1.57 and 2.50 times the total blood volume were recovered from the feces of lambs 11 and 12, respectively, during the entire period of infection. The difference in these figures may be due to the fact that the onset of hemorrhage in lamb 11 following the administration of the single large dose of larvae was more sudden and the loss of blood more rapid than in lamb 12, which received smaller doses of larvae over a

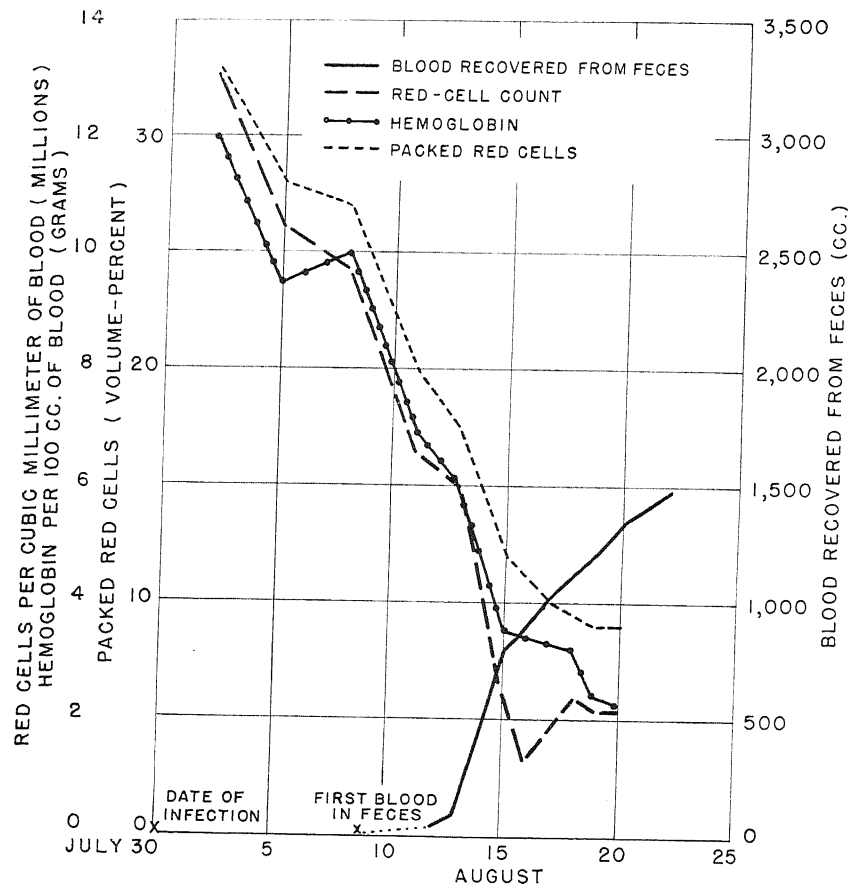


FIGURE 5.—Red cells and hemoglobin in the blood of lamb 11 correlated with the quantity of blood recovered from the feces passed after experimental infection with *Haemonchus contortus*.

period of time. In the second lamb the loss of blood was apparently less rapid at first. This circumstance permitted a greater restoration of the blood lost than was possible in the first lamb. The slower hemorrhage would therefore result in the loss of a greater volume of blood while producing the same degree of anemia as in lamb 11.

Although the quantitative relationship between the volume of blood recovered from the feces and the volume of blood lost by hemorrhage into the abomasum is at present unknown, since losses from digestion,

assimilation, and bacterial decomposition undoubtedly occur, such losses would tend to increase rather than to decrease the quantity of blood at the source of hemorrhage represented by a unit quantity of hemoglobin recovered from the feces. Therefore, any error involved in the determination due to this factor would not invalidate the conclusions drawn from the present analyses.

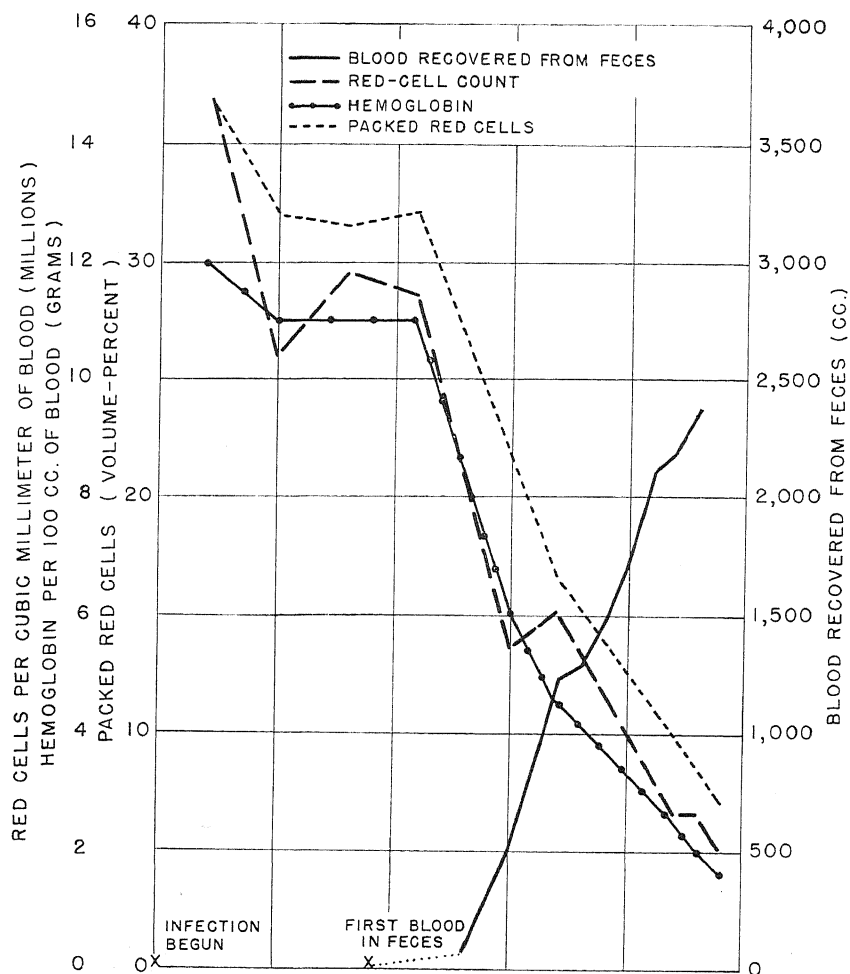


FIGURE 6.—Red cells and hemoglobin in the blood of lamb 12 correlated with the quantity of blood recovered from the feces passed during experimental infection with *Haemonchus contortus*.

As already stated, lamb 17, which was used as a control in this phase of the work, was bled daily, except Sundays, from the jugular vein. The data obtained are plotted in figure 7. As is shown in the figure, 3,560 cc. of blood were removed from this lamb during a period of 15 days. At the end of that time the animal had slightly more than 2 million red cells per cubic millimeter of blood, a stage of anemia



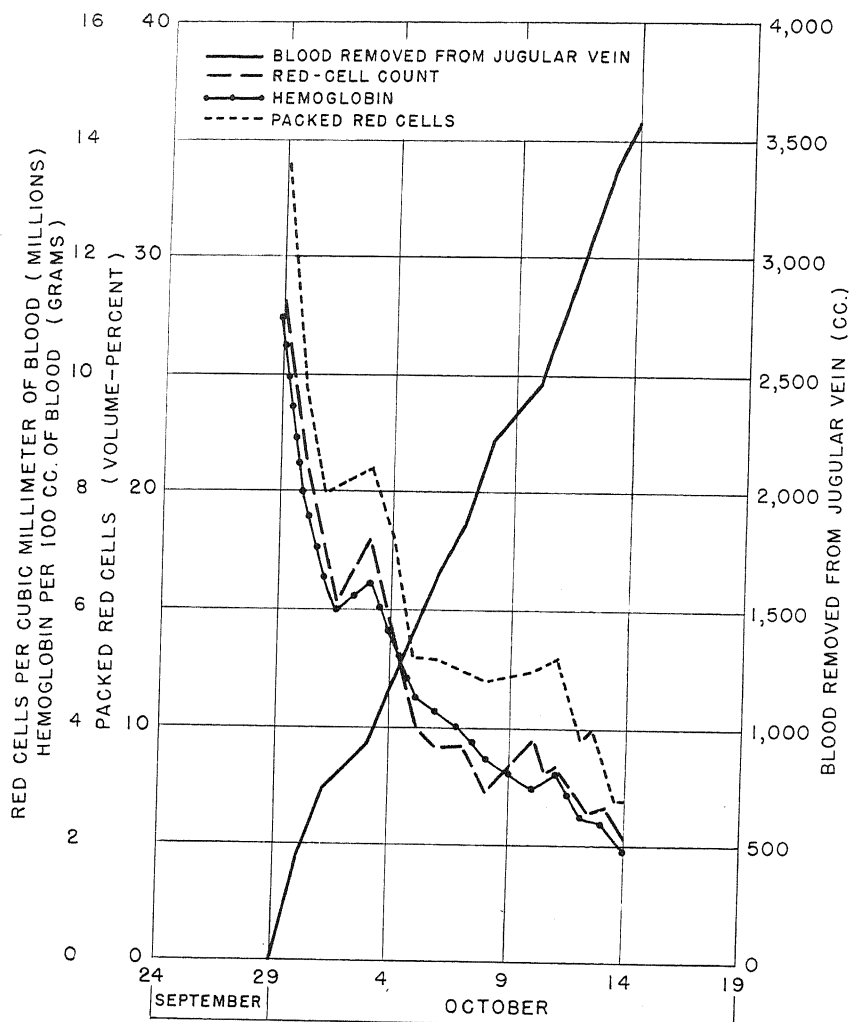


FIGURE 7.—Red cells and hemoglobin in the blood of lamb 17 correlated with the quantity of blood removed from the jugular vein.

comparable to that in lambs 11 and 12. Lamb 17 weighed 38 pounds, and its total blood volume was 1,444 cc., according to Hodgson (5). About 2.47 times its blood volume, therefore, was removed in producing this degree of anemia by the end of the 15-day period during which it was bled. When an allowance is made for the replacement of lost blood during the two Sundays that lamb 17 was not bled, the quantities of blood lost in the three lambs (Nos. 11, 12, and 17) agree sufficiently well to show that the anemia in stomach worm disease in sheep is definitely caused by hemorrhage.

#### CONTROL LAMBS

The average values for the number of red cells per cubic millimeter of blood, the quantity of hemoglobin per 100 cc., and the volume

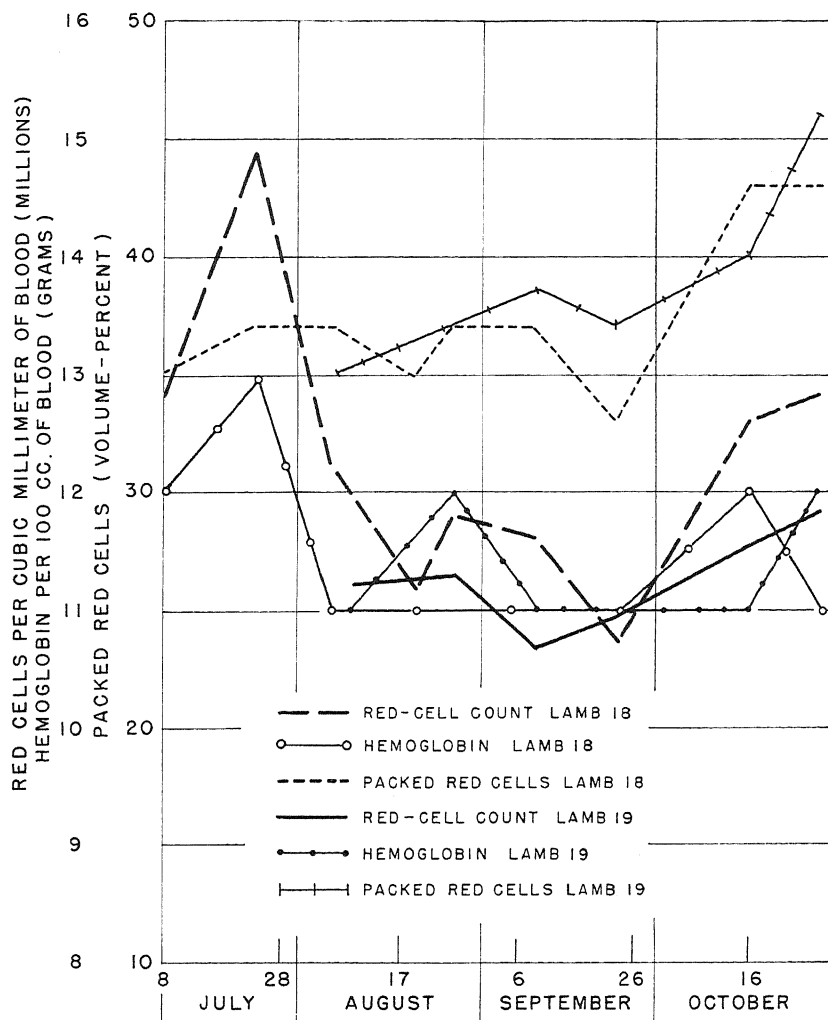


FIGURE 8.—Red cells and hemoglobin in the blood of control lambs 18 and 19.

percentage of packed red blood cells for the control lambs 18 and 19 are given in figure 8. These data show that the blood of the two lambs remained normal in these respects during the entire time that they were under observation.

#### RETICULOCYTE COUNTS

The infected lambs 9, 11, and 12 and the bled lamb 17 were considered to be typical of all the infected lambs in number of reticulocytes per 1,000 red cells. Therefore, these animals were selected for this phase of the study. The results are shown in table 2.

TABLE 2.—Number of reticulocytes per 1,000 red cells in three lambs infected with *Haemonchus contortus* and in lamb 17, bled from the jugular vein

Lamb 9		Lamb 11		Lamb 12		Lamb 17	
Date	Reticu- lyocytes	Date	Reticu- lyocytes	Date	Reticu- lyocytes	Date	Reticu- lyocytes
	<i>Number</i>		<i>Number</i>		<i>Number</i>		<i>Number</i>
Aug. 26.....	0	Aug. 2.....	0	Aug. 2.....	0	Sept. 29.....	0
Aug. 28.....	0	Aug. 8.....	0	Aug. 8.....	0	Sept. 30.....	0
Sept. 4.....	0	Aug. 11.....	0	Aug. 11.....	0	Oct. 1.....	0
Sept. 13.....	0	Aug. 13.....	0	Aug. 13.....	0	Oct. 3.....	15
Sept. 21.....	21	Aug. 17.....	0	Aug. 17.....	0	Oct. 4.....	44
Sept. 28.....	31	Aug. 18.....	83	Aug. 18.....	0	Oct. 5.....	41
Oct. 9.....	2	Aug. 19.....	160	Aug. 19.....	5	Oct. 6.....	55
Oct. 15.....	0	Aug. 20.....	80			Oct. 7.....	76
Oct. 21.....	11			Aug. 22.....	13	Oct. 8.....	80
Oct. 29.....	2			Aug. 23.....	23	Oct. 10.....	86
Nov. 5.....	11			Aug. 24.....	50	Oct. 11.....	100
Nov. 15.....	0					Oct. 12.....	95
Nov. 24.....	0					Oct. 13.....	129
Dec. 3.....	0					Oct. 14.....	75

As is shown in table 2, there was a great variation in the number of reticulocytes found in the blood of the four lambs at different times during the development of the anemia. This variation was apparently due to the individual reaction of each lamb to the suddenness and severity of the experimental infection. Since the number of reticulocytes in lamb 11 was even higher than that in lamb 17, there appeared to be no evidence that hematopoiesis was interfered with by toxins of any kind. No reticulocytes were found in the blood of the control lambs.

#### TOTAL AND DIFFERENTIAL WHITE-CELL COUNTS

The data on the total and differential white-cell counts for the experimental and control lambs are shown in table 3.

Statistical analysis of the data in table 3 indicated the following significant differences. The number of white cells per cubic millimeter of blood in each group of experimental lambs was greater than that of the controls, thus indicating that the same changes in the total white-cell count occurred when hemorrhage was the sole cause of the anemia as when it was brought about by the *H. contortus* infection. The lambs dying from infection and the bled lamb had higher percentages of polymorphonuclear cells than the controls. The first-mentioned group also had more polymorphonuclear cells than the lambs that recovered from the infection. The group that recovered, on the other hand, had a higher percentage of eosinophiles than the controls and fatal cases, a finding which indicated a positive correlation between eosinophiles and the development of resistance to the infection. The percentages of basophiles and monocytes were so small that no conclusions could be drawn from the observations. The differential counts of the lambs that died from the infection and those of the bled lamb were practically the same.



TABLE 3.—Average total and differential white-cell counts of the lambs

Lamb group	Lamb No.	Total white-cell counts made	Total white-cell counts per cubic millimeter of blood	Differential white-cell counts made	Lymphocytes	Polymorpho-nuclears	Eosinophiles	Basophiles	Monocytes	Smudges
		Num-ber	Thou-sands	Num-ber	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Recovered from disease	3	15	7.81	14	75.64	22.40	1.37	0.44	0.00	0.15
	4	13	9.63	13	87.87	14.58	3.05	.32	0.	.27
	9	13	6.28	14	62.87	35.96	.69	.18	.11	0
	Average		7.91		75.16	24.55	1.67	.31	.04	.13
	Standard deviation		±14.10		±82.00	±84.86	±11.91	±4.23	±0.64	±1.93
Died from infection	5	5	9.36	5	63.30	36.50	.10	.10	0	0.0
	11	11	8.74	7	69.28	30.56	.14	.03	0	0
	12	10	10.69	9	69.56	29.85	.15	.05	0	.37
	Average		9.61		67.98	31.67	.13	.06	0	.16
	Standard deviation		±11.05		±47.21	±48.56	±1.15	±0.74	0	±2.27
Bled from jugular vein	17	14	8.09	14	69.15	30.54	.09	.00	.09	.07
	Standard deviation		±4.70		±25.32	±24.53	±0.57	0	±.81	±1.00
Controls	18	9	6.36	7	81.67	16.51	.41	.84	0	.54
	19	6	7.13	6	74.50	23.83	.42	.52	.23	.50
	Average		6.67		78.37	19.89	.41	.69	.11	.52
	Standard deviation		±3.26		±24.25	±23.91	±1.50	±3.26	±.69	±3.55

## PATHOLOGICAL FINDINGS

Evidences of hemorrhage produced in the abomasum by *Haemonchus contortus* have been repeatedly observed by the author on lambs at autopsy. However, in order to clarify the pathological picture produced by this nematode and relate it to the life history of the worm, the following observations were made on lambs 13, 14, 15, and 16, which were given large doses of infective larvae and were then killed at different intervals after infection (table 1). Four days after infection the larvae were found between the papillae of the mucosa of the abomasum. There was no evidence of injury to the mucosa nor of hemorrhage, and the larvae had not attached themselves to the mucosa. Seven days after infection the surface of the mucosa of the abomasum was covered with blood coagula and mucus (pl. 1, *H*). The young, fourth-stage larvae were found under these coagula next to the mucosa but apparently were not attached to it. In 11 and 23 days after infection, the aspect of the stomach had changed little except that both the larvae and the coagula had increased in size and the quantity of blood within the stomach had increased markedly. Profuse hemorrhage was present in the abomasum of lamb 16.

Pathological observations also were made on lambs 11 and 12, which died as a result of infection, and on lamb 18, a control. Plate 1, *C*, shows lamb 12 a few minutes before death. This lamb, as contrasted with control lamb 18 (pl. 1, *A*), was noticeably weak and unable to hold up its head or to stand firmly on its four feet. *F* shows the paleness of the white of the eye of lamb 12, caused by the severe anemia. *A*

normal eye is shown in *D*. *I* shows some of the viscera of lamb 12 at autopsy. In contrast to *G*, which shows the organs of control lamb 18, the viscera of lamb 12 have a bloodless appearance in spite of the fact that the lamb was not bled out prior to necropsy. All these illustrations show the severe anemia that is characteristic of *H. contortus* infection in lambs.

Plate 1, *E*, shows the abomasum of lamb 12 with many hundreds of young adult *Haemonchus contortus* attached to the mucosa in the pyloric region. This localization of the worms is unusual as they are usually found scattered over the entire mucosa.

Sections of the stomach, liver, heart, lung, kidney, and spleen of lambs 11 and 12 were sent to the Pathological Division of the Bureau of Animal Industry for histological examination. The report on these organs indicated some slight degenerative changes in the heart muscle, liver, and kidney, which may have occurred post mortem, and some pneumonic changes in the lungs, due to causes other than the nematode infection. The spleen and stomach, which are of particular interest in this instance, were described as follows:

**Spleen.** There was comparatively little blood remaining in the splenic sinuses, and the rather limited quantity of blood throughout the splenic pulp, as compared with the normal, is indicative of anemia of this organ.

**Stomach.** Sections from the stomach showed extensive sloughing of the mucosa, and masses of cellular debris could be seen deposited on the surface of the remaining intact mucosa. There was no congestion, and erythrocytes were present only in limited numbers. Lymphoid cells were present in rather large numbers and these cellular elements, together with limited numbers of plasma cells and eosinophiles, could be seen invading various portions of the mucosa. Slight fibroblastic proliferations were also noted in sections. The submucosa and muscularis showed evidence of edema. Sections of stomach worms were seen in close contact with the surface of the mucosa, and a number of the parasites had penetrated, or burrowed beneath the surface and were partly or completely embedded in the upper third of the mucosa. The stomach lesions [pl. 1, *B*] found in lambs 11 and 12, give evidence that these parasites are capable of causing considerable damage to the mucosa, particularly when present in large numbers. Such injury is doubtless sufficient to cause more or less continuous capillary hemorrhage, resulting eventually in extreme anemia and death of the animal.

These data, therefore, support the conclusion suggested by the data given previously in this paper that the anemia of *H. contortus* infection in sheep is due to hemorrhage.

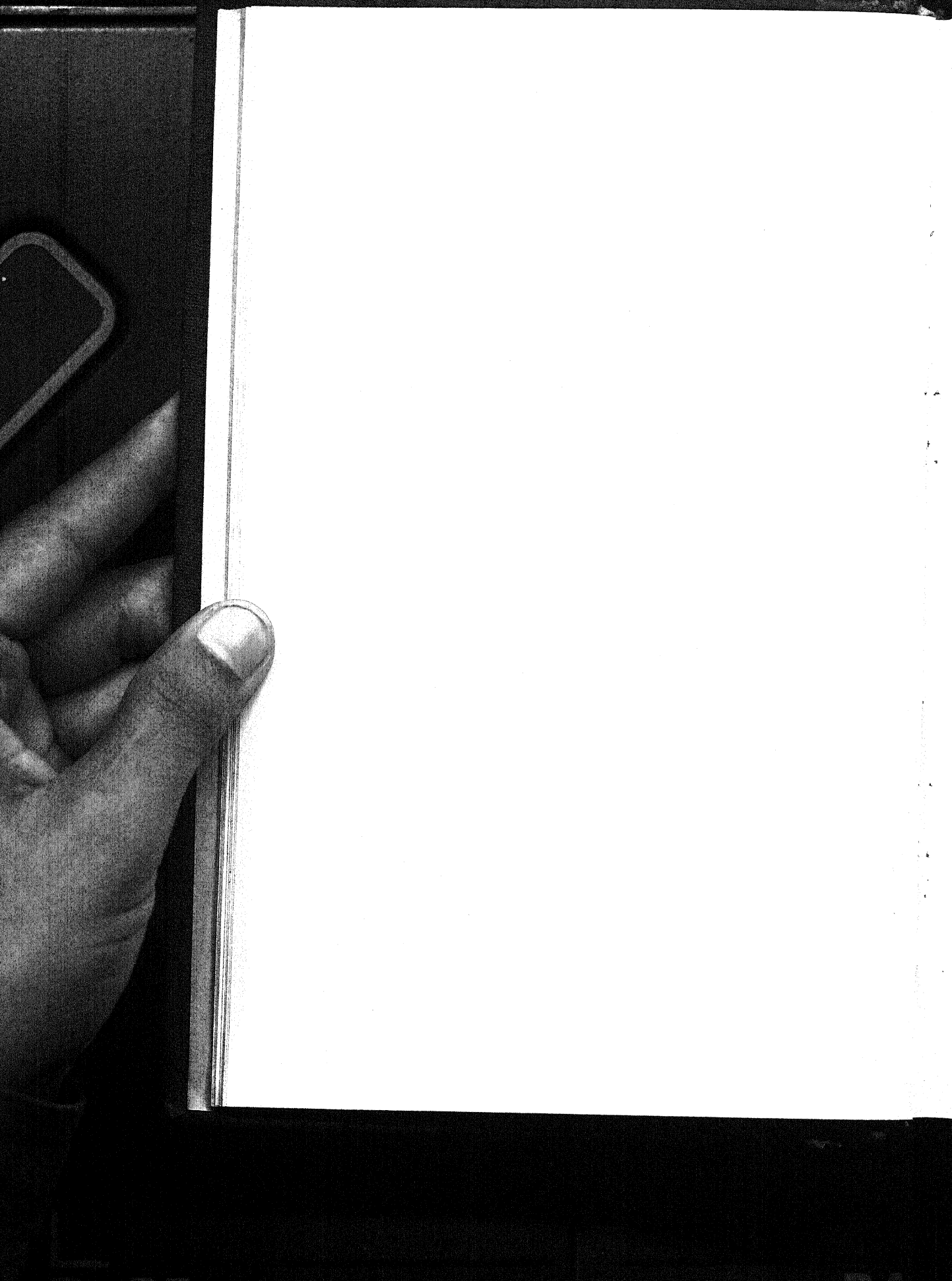
#### DISCUSSION

The observations reported in the present paper on the hematology of *Haemonchus contortus* infection in sheep agree in general with the findings of other workers. The pathological changes found in the abomasum and associated with the infection were similar to those described by Veglia (8) and Fourie (4) except for the absence of the gross ulcerations described by the latter. The histological examination of the organs of lambs dying of the infection, however, did not disclose the peculiar crystals in the liver described by Fourie, but simply gave evidence of the injuries produced by the nematodes in the wall of the fourth stomach, of the capillary hemorrhage resulting therefrom, and of the extreme anemia.

The conclusions of other workers concerning the possible direct relation between the anemia associated with *H. contortus* infection in sheep and gastric hemorrhage have been given added validity by the



Pathological findings in some of the experimental lambs: *A*, Uninfected control lamb 18; *B*, section of stomach of lamb 12 showing destruction of the mucosa; *C*, lamb 12 shortly before death from stomach worm infection; *D*, eye of normal uninfected lamb; *E*, stomach of lamb 12 showing young adult of *Haemonchus contortus* (dark areas in lower right portion of photograph) attached to the pyloric region; *F*, eye of lamb 12, showing paleness of white; *G*, viscera of uninfected control lamb 18; *H*, stomach of lamb 14 killed 7 days after infection; *I*, viscera of lamb 12, bloodless in appearance in contrast with normal viscera.





results of the analyses for blood in the feces of infected sheep, made during the present investigation. The conclusions drawn from this work were similar to those arrived at by Wells (9), who calculated that in dogs 1,000 hookworms could produce a loss to the host of 360 cc. of blood per day, a loss which, if continued, would result in a severe or fatal anemia. Foster and Landsberg (3) later came to the conclusion that the presence of a toxin was not necessary to account for the anemia of hookworm disease in dogs, since the anemia was like that associated with chronic hemorrhage.

#### SUMMARY

In a study involving *Haemonchus contortus* infection in sheep, 19 crossbred Hampshire-Southdown lambs 2 to 8 months old were used. The work was carried on at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., in 1933 and from 1936 to 1938, inclusive.

From 2,000 to 181,000 infective larvae of *H. contortus* were administered to lambs in single and multiple doses. Although one dose of 35,000 larvae undoubtedly would have produced death in lamb 5 had this animal been allowed to die naturally, much larger total doses were not fatal when administered in small daily doses. This observation indicates the development of resistance to infection in the lambs receiving the multiple doses.

The data show that *H. contortus* produced a severe and sometimes fatal anemia in sheep. Blood appeared in the feces 6 to 10 days after infection. In the two cases which were fatal, blood was present in the feces until the death of the animals. In lambs recovering from the infection the anemia was negatively correlated with the number of worm eggs per gram of feces. In lambs dying before the worms matured anemia developed rapidly, but a positive diagnosis of the infection was impossible before autopsy owing to the absence of worm eggs in the feces. In two of the fatal cases, no worm eggs were passed in the feces, whereas one of the nonfatal cases had an egg count of 24,800 eggs per gram of feces. Accordingly, there was no correlation between the number of eggs per gram of feces and the fatality of the infection.

The quantities of blood lost during a period of 10 days in two of the lambs that died were 1,492 and 2,380 cc., or 1.57 and 2.50 times, respectively, the original quantity of blood in the lambs, as calculated from the body weight of the animals. These quantities compared favorably with the 3,560 cc. of blood taken from the jugular vein of a healthy lamb over a period of 15 days, until approximately the same degree of anemia had been produced. This volume of blood was about 2.5 times the original volume of blood in this lamb, as calculated from its body weight. That the anemia associated with *H. contortus* infection in sheep was due to gastric hemorrhage alone was further indicated by the normal icteric index, the presence of reticulocytes in the blood of the anemic lambs, and the gross pathology and histological findings.

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## ARRANGEMENT OF THE TISSUES BY WHICH THE COW'S UDDER IS SUSPENDED <sup>1</sup>

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### INTRODUCTION

The broken-down, pendulous udder is a defect that occurs frequently among high-producing dairy cows. There are a number of different types of broken-down udders. Some become detached from the body wall, so that the hand can be inserted between the abdomen and the upper surface of the front quarters. Some become elongated vertically like a sack and have a tendency to swing from side to side as the cow walks. In some the median support appears to undergo a relaxation, permitting the floor of the udder to sag and causing the teats to point outward from the sides of the udder. In others the rear attachment appears to become lax, allowing the udder to swing forward, and sometimes causing the rear quarters to occupy a position lower than the front quarters. These are the more common types of broken-down udders; there are many variations.

Generally, this breaking down is a condition that occurs with large, heavy udders. Usually it becomes progressively more pronounced with advance in age. Occasionally it occurs with udders of moderate size and may be pronounced early in life.

The breaking down of udders is frequently attributed to heavy milk production. The physiological function of milk secretion has been developed by breeding and improved nutrition until it has reached amazingly high levels. The range cow produces only enough milk to raise her calf. The good dairy cow produces in 1 year an amount of milk that is equivalent to 8 to 12 times her live weight. In some cases cows have produced an amount of milk in 1 year that was equivalent to 20 times their live weight. The combined weight of the udder and the milk that it contains is often very great.

The broken-down or pendulous udder is objectionable for a number of reasons. Such udders are difficult to milk; they are hard to keep clean; they interfere with the cow's locomotion; they are subject to friction, trauma, and injuries from sharp objects; and the teats are often injured as a result of being stepped on by the cow herself or by cows in adjoining stalls. Moreover, such udders are unsightly. Good-producing cows that have pendulous udders have much lower sale value than cows with well-attached udders, although little is known of the effect of this condition on the animal's producing ability. In view of the greater liability of the pendulous udder to injury such an udder would appear to be more subject to mastitis.

A more comprehensive knowledge of the supporting tissues of the udder may suggest methods of management that will be helpful in preventing broken-down udders; it may suggest points to be consid-

<sup>1</sup> Received for publication July 17, 1941.

ered in the selection of cows that are less likely to be susceptible to this trouble; or it may suggest points to be considered in the selection of animals for a breeding program having for its objective the fixing of an inheritance for strongly supported udders. With these facts in mind a study was undertaken to determine the nature of the structures that support and maintain the udder in well-balanced suspension. The results of the study are presented in this paper.

#### REVIEW OF LITERATURE

A recent tabulation of the weights (after milking) of excised udders of cows that were slaughtered by the Bureau of Dairy Industry, in connection with its studies of conformation and anatomy in relation to producing capacity at Beltsville, Md., gives some idea of the size of the udder of high-producing dairy cows.<sup>2</sup> Data were available for 50 lactating Holstein-Friesian cows 4 years of age or over. On an average the weight of the udder declined with advance in the stage of lactation. The udders of 17 cows that had been milked 3 months or less in the current lactation at the time of slaughter averaged 72.98 pounds; those of 25 that had lactated 6 months or less averaged 65.07 pounds; those of 35 that had been milked 9 months or less averaged 58.08 pounds; those of 41 that had been in milk for 12 months or less averaged 55.89 pounds; and the entire group of 50 udders had an average empty weight of 52.47 pounds. Among these there was 1 that weighed 165.65 pounds, which was approximately 11 percent of the live weight of the cow; and 2 others that weighed 138.20 and 101.85 pounds, respectively.

The results of a number of investigations indicate the weights of blood and milk that the udder may carry.

Swett, Miller, and Graves (8)<sup>3</sup> found that a large proportion of the milk obtained at a milking is stored in the udder before the milking process is commenced. Thus a heavy-milking cow producing 100 pounds of milk on twice-a-day milking might have 50 pounds or more of milk in the udder before each milking.

Shaw and Petersen (6) found that for each pound of milk secreted, nearly 400 pounds of blood passes through the udder. This means that for a cow producing 100 pounds of milk daily, the blood passing through the udder in that length of time would amount to approximately 40,000 pounds. The total quantity of blood in the body of a lactating cow has been determined (2, pp. 54-55) to be approximately 8 percent of the cow's live weight. This means that the bodies of few cows would contain more than 150 pounds of blood. Presumably only a small proportion of the total amount of blood would be present in the udder at any time. According to Kay (5) the udder in full lactation takes about one-quarter of the heart's output of arterial blood.

Thus the total weight of the milk and blood in the udder at any time might easily be 50 to 60 pounds, but probably would not exceed 75 pounds, except in unusual cases. This would mean that the total weight of a large, heavy-producing udder, together with its contents, might vary between limits of approximately 100 to 250 pounds. In view of the great weight of the functioning udder and its contents it is

<sup>2</sup> Unpublished data.

<sup>3</sup> *Italic numbers in parentheses refer to Literature Cited, p. 43.*



not surprising that in many cases the anatomical structures by which the udder is suspended are inadequate to support it in its proper position.

There are some indications that the tendency for the udder to break away from its attachments is inherited. It appears to occur with high frequency among the daughters of certain sires.

The weakness may result from poor tonus in the supporting structures. According to Hammond (4) the formation of pendulous udders is the result of excessive and continued internal pressure produced by the accumulated milk in the udder.

Some believe that a weakness in the support of the udder causes faulty circulation which tends to produce stasis, edema, fibrosis, and an increase in the size and weight of the udder. This in turn would further stimulate the breaking-down process and make the abnormalities resulting from faulty circulation even more pronounced.

Sisson (7, p. 620) describes the suspensory apparatus as follows:

The mammary glands, normally two in number, are popularly termed the udder. They are very much larger than in the mare, and the body of each is somewhat ellipsoidal in form, but flattened transversely. The base of each gland is slightly concave and slopes obliquely downward and forward in adaptation to the abdominal wall, to which it is attached by means of a well-developed suspensory apparatus (Lig. suspensorium mammarium) which extends backward and is attached to the symphysis pelvis by means of the strong plate of tendinous tissue (Tendo subpelvina). This plate of tissue attaches the prepubic tendon to the ventral part of the symphysis. The suspensory apparatus consists essentially of four sheets of tissue two of which are well developed and median in position and are chiefly yellow elastic tissue; the two glands are separated by this double septum which attaches to the medial flat surface of each gland. The lateral sheets (containing less elastic tissue), arise from the subpelvic tendon posterior to the udder; on reaching the abdominal floor they diverge and pass laterally to the external inguinal ring. They extend downward over the udder and divide into superficial and deep layers: the superficial layer attaches to the skin where it reflects off the udder to the medial face of the thigh, and the deep layer is thicker and attaches to the convex lateral surface of the udder by numerous lamellae which pass into the gland. It is in relation posteriorly to the large supramammary lymph-glands and a quantity of fat.

Bitting (1) was one of the first in this country to publish results of anatomical studies of the suspensory apparatus of the cow's udder. His description was general and included nothing of importance that is not covered by that of Sisson (7), except that he emphasized the importance of the strength of the abdominal wall as a factor in determining the shape and apparent size of the udder. He states (1, p. 39):

In a cow with loose abdominal walls, dropping directly down from the pubis, thus forcing the udder downward and backward, the organ will appear to be much larger than in one in which the walls are stronger. This sometimes accounts for the apparently sudden development of a good udder after the second or third calf. The muscles of the abdomen become more relaxed and the udder becomes more pendulous.

An interesting and valuable contribution to the subject was made by Emmerson<sup>4</sup> who dissected several cow udders and described and illustrated many of the supporting structures. Emmerson elaborated considerably with regard to the lamellae (plates) which are given off by the deep faces of the lateral sheets, and penetrate the glandular tissue in a medioventral (inward and downward) direction to become incorporated in the interstitial framework of

<sup>4</sup> EMMERSON, M. A. STUDIES OF THE MACROSCOPIC ANATOMY OF THE BOVINE UDDER AND TEAT. 1928. [Unpublished thesis. Copy on file in library of Iowa State Col. of Agr. and Mech. Arts, Ames.]

the udder. He also pointed out that the lateral suspensory ligamentous sheets continue to the ventral (lower) border of the gland at which point the lamellae anastomose and become continuous with those of the sheets of elastic tissue which make up the median septum, thus forming a sling or cradlelike structure in which the udder is held. Unfortunately the results of Emmerson's work are not available in published form. However, his work has been quoted rather freely by Turner<sup>5</sup> and by Espe (3, pp. 9-10).

There is no question as to the accuracy of Sisson's brief description of the udder's suspensory structures, or of Emmerson's elaboration concerning them. To the anatomist they are, without doubt, understandable and adequate. However, an amplified description expressed in simpler terminology and liberally illustrated should enable the research worker, the cattle judge, the dairy farmer, and all others interested in dairy cattle problems, to visualize and understand more clearly the nature of mammary-gland suspension in the cow.

In evaluating the udder capacity of living cows it would be desirable to be able to calculate the volume of the udder. To do this it is necessary to have a knowledge of the shape of the dorsal (upper) surface of the udder, and of the curvature and degree of slope of that part of the abdominal wall to which the fore udder is attached. It has been pointed out by the authors (see 9, p. 9) that the rear and front quarters of the udder generally produce in the ratio of 3 to 2 (58.2 percent from the two rear quarters and 41.8 percent from the two front quarters), and that a similar ratio existed between the depth of the rear and front quarters of the excised udders studied (10.29 inches for the rear and 6.38 inches for the front). It is well known, of course, that the abdominal wall is lower at the anterior attachment of the udder than at more posterior points and that it curves upward toward the rear. But the shape of the curve of the abdominal wall from front to rear, or from side to side, has not been readily or accurately measured in the living cow. Neither has the outline of the dorsal surface of the udder been accurately ascertained.

#### PLAN OF STUDY

In order to obtain additional information that might throw light on the problems of the broken-down or pendulous udder in heavy-producing cows, and also to obtain data that might make it possible to calculate the volume of udder of the living cow, a plan was outlined for studying in detail the structures suspending the udder of a cow that was known to be a good producer. Because of the desirability of obtaining photographs of dissections of these structures in their normal position, the plan called for the work being done with the animal in a standing position. This was accomplished by following the methods that are later described.

#### HISTORY AND CHARACTERISTICS OF COW USED

Holstein-Friesian cow No. 1216 was selected for this study. She was 5 years 10 months of age and had been in milk for 7 months of the fourth lactation period when slaughtered. Her live weight, 4 months before slaughter, was 1,320 pounds. Her production record at 3 years 2 months of age was 14,734 pounds of milk and 540 pounds

<sup>5</sup> TURNER, CHAS. W. THE COMPARATIVE ANATOMY OF THE MAMMARY GLANDS WITH SPECIAL REFERENCE TO THE UDDER OF CATTLE. 378 pp., illus. Columbia, Mo. 1939. [Mimeographed.] See pp. 31-37.

of butterfat (equivalent to 17,533 pounds of milk and 643 pounds of butterfat at maturity).

The mammary-gland development of cow No. 1216 was slightly retarded until she was 2 months of age. From 3 to 5 months of age it was normal for the breed, and from 6 to 18 months it was definitely superior to the breed average. Up to 4 months of age there was a tendency for the attachments of the gland tissue to the body to be inferior. Subsequently, up to 18 months, the attachments were superior to the average. However, the attachment of the udder of this animal, both as a heifer and as a lactating cow, was poorer at the rear than at the front at nearly every examination. This was particularly noteworthy at the last examination, 3 days before slaughter, when she was given grades of 5 and 3+ respectively for front and rear attachments of gland tissue, when graded on a 9-point basis, in which 9 is excellent, 5 average, and 1 extremely poor. The gland tissue hung low in the rear quarters before slaughter and the separation between right and left halves was marked. During lactating life the udder was of fair shape and medium or above in size. The weight of the udder was not obtainable because of the method employed in slaughtering the cow and in preparing her for anatomical study. The

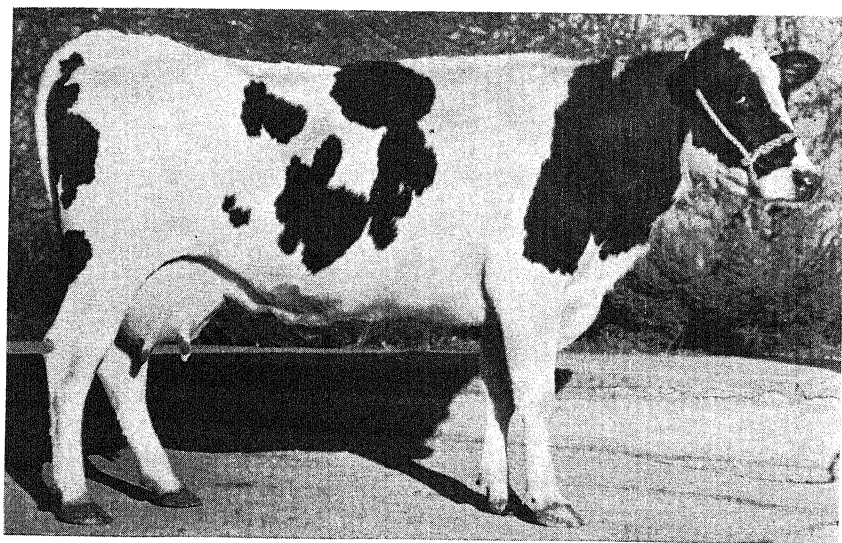


FIGURE 1.—Cow No. 1216, as she appeared about 1 month before she was killed.

udder was of medium quality and not excessively fibrous or meaty. It was somewhat inclined to be pendulous although this condition was not extreme.

Strip-cup examinations of the milk showed that this cow had had numerous flare-ups of mastitis of varying severity but of short duration. Mastitis was noted in all four lactation periods except the second. During both the first and third lactation periods all the quarters of the udder were affected at one time or another. Swelling and edema were noted prior to first calving, but neither was extreme. All of the edema had disappeared 15 days after calving and nearly all of the swelling was gone at the end of the first month. It is considered



improbable that either the mastitis or the swelling was of sufficient severity or duration to affect the suspensory structures or the attachments of the udder.

#### PROCEDURE FOLLOWED

The method generally employed in preparing large animals for dissection was used in this study. On the morning of January 25, 1939, the cow was rendered insensible by the injection of chloral hydrate and the subsequent administration of chloroform. She was then bled from an incision in the right carotid artery until heart action ceased, after which some residual blood was drawn by vacuum. The carotid artery and other severed blood vessels were tied off, and a glass tube, connected by a rubber hose to a tank located at an elevation some 12 or 14 feet above the cow, was inserted in the jugular vein and tied securely in position. By this means the filling of the circulatory system of the cow was commenced. A formalin solution of approximately 10-percent strength was used to preserve and harden the tissues. Meanwhile a heavy hook was inserted beneath the sacrum, and two smaller ones were inserted on opposite sides of the skull somewhat below and slightly posterior to the eyes (beneath the cheek bones or zygomatic processes of the malar bones). By means of ropes attached to these hooks the cow was hoisted into a standing position. An additional support in the form of a timber placed crosswise under the floor of the chest aided in holding the midportion of the cow at the proper height. A stoneboat was placed under the cow, the feet and legs of the cow were pulled into positions as nearly normal as possible, and each hoof was nailed to the stoneboat.

With the cow in this position, which was essentially normal except for the outstretched head, the filling of the circulatory system was continued. Within the first 3 hours about 30 gallons of formalin flowed into the jugular vein, and an additional 10 gallons was nearly exhausted some 3 hours later. During the course of the filling the udder became distended and many of the subcutaneous veins on the legs and under part of the body stood out prominently. Residual milk in the udder commenced to flow from the teats, and teat plugs were inserted. Incisions made for the supporting hooks were packed and sewed together to prevent escape of the formalin, and very little was lost. Some formalin was poured into the nostril to prevent or minimize fermentation in the rumen. Measurements of the circumference of the paunch on January 26 and 27 were 248 cm. and 247 cm., respectively. This lack of increase indicated that excessive fermentation had not occurred.

On January 27 a photograph was made showing the embalmed cow with all supports in place. The supporting apparatus was removed and the cow was again photographed. There was no significant change in the position of the cow, which showed that the tissues were adequately preserved and hardened to provide ample rigidity. The supporting equipment was replaced, however, to keep the embalmed body in an unchanged position until dissection was made. Figure 1 shows the appearance of the living cow about 1 month before she was killed. Figure 2, *A*, shows the embalmed, hardened cadaver standing, with the supporting mechanism and injection tube in position; and figure 2, *B*, shows the cadaver in the same position unsupported.

Mention has been made of the comparatively poor rear attachment of the udder which occurred in this animal as a calf and during lac-



tating life, and which was particularly noteworthy at an examination about a month before she was slaughtered. Figure 3, *A*, shows a rear view of the udder of the living cow, and figure 3, *B*, shows its appearance after she had been embalmed and hardened in situ.

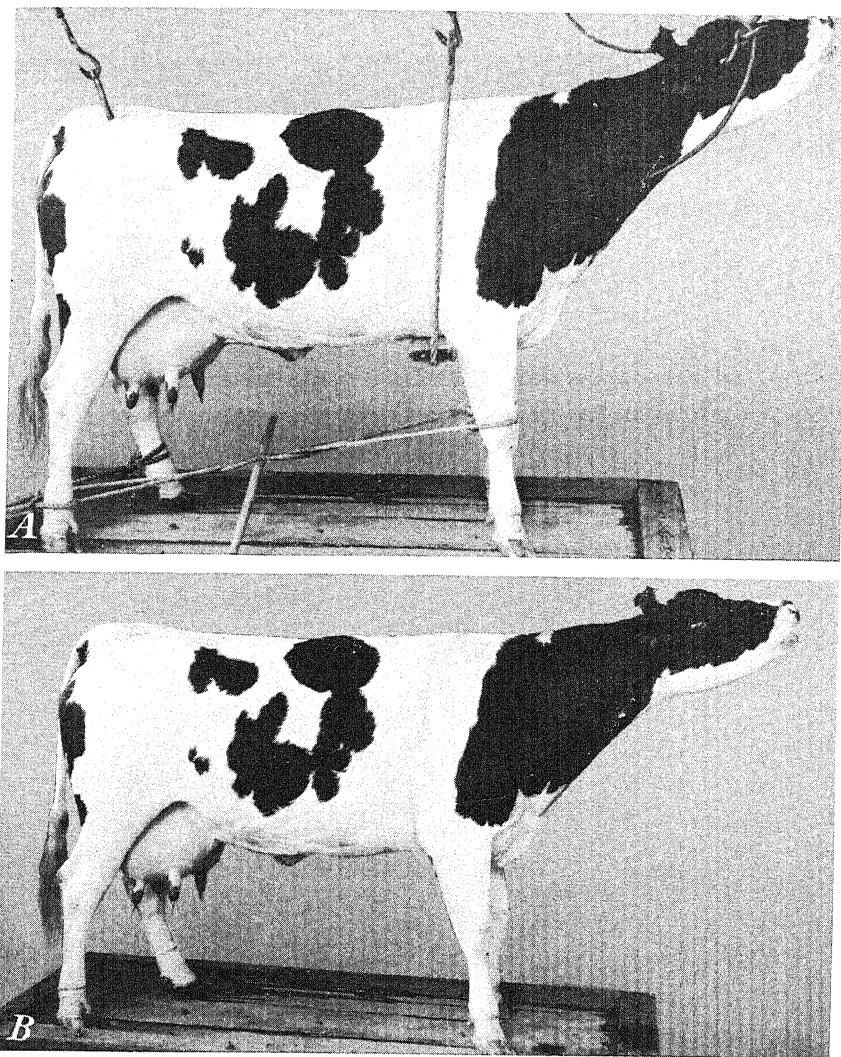


FIGURE 2.—The embalmed cadaver: *A*, With supporting equipment and injecting tube in position; *B*, with supporting equipment and tube removed.

Dissection was commenced on January 30. Photographs were taken as the work progressed and as one after another of the tissues by which the udder was suspended were uncovered. The dissecting and photographing work was carried on with the embalmed cow standing as shown in figure 2, *A*, with the hooks attached and the hoisting apparatus taut to prevent a change of position.

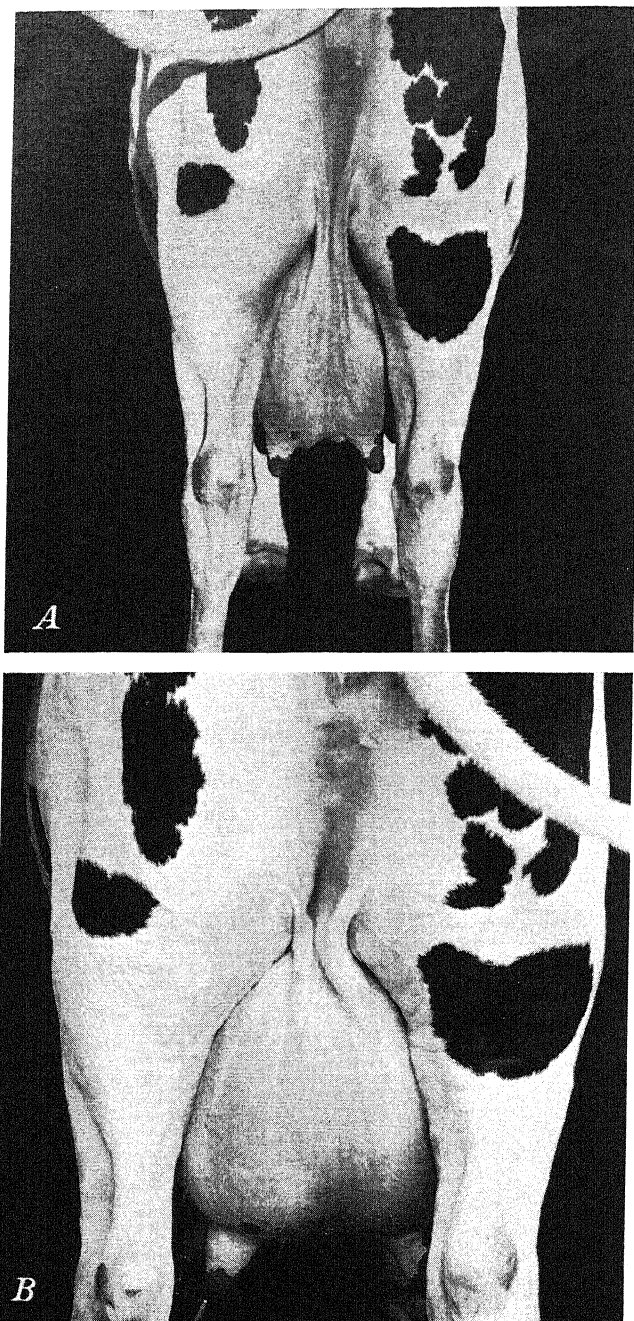


FIGURE 3.—*A*, Rear view of the udder before the death of the cow; *B*, its appearance after being embalmed.

## PRESENTATION AND DISCUSSION OF RESULTS

At this point it may be helpful to list and number in the order found the principal parts of the suspensory apparatus of the udder, in order that they may be identified more easily by referring to the tissue number in the discussion of the step-by-step dissections by which they were brought to light. The principal parts of the suspensory apparatus are as follows:

*Tissue No. 1.*—The skin, which serves in a minor capacity to suspend and stabilize the udder.

*Tissue No. 2.*—The fine areolar, subcutaneous tissue (superficial fascia), which serves as an attachment between the skin and the underlying tissues.

*Tissue No. 3.*—The cordlike (coarse areolar) tissue, which forms a loose bond between the dorsal (upper) surface of the front quarters of the udder and the abdominal wall.

*Tissue No. 4.*—The pair of superficial layers of the lateral sheets of partly elastic tissue (lateral suspensory ligaments), which arise from the subpelvic tendon, extend downward and forward over the udder, and reflect off the udder to the medial (inner) face of the thigh.

*Tissue No. 5.*—The pair of deep, somewhat thicker layers of the lateral sheets, which have the same origin, extend downward over the udder and virtually envelop it, but which, unlike the superficial layers, attach to the convex lateral surfaces of the udder by numerous lamellae (plates) which pass into the gland and become continuous with the interstitial framework of the udder.

*Tissue No. 6.*—The subpelvic tendon. The tendon itself, strictly speaking, is not a part of the suspensory apparatus of the udder. Nevertheless it plays an important role as it gives rise to both the superficial and deep layers of lateral sheets described above.

*Tissue No. 7.*—The two adjacent sheets of heavy yellow elastic tissue, median in position, which arise from the abdominal wall, and attach to the medial flat surfaces of the two glands to form a double septum between them (median suspensory ligament).

A wide divergence of opinion exists with regard to the relative importance of these different parts of the suspensory apparatus. Emmerson<sup>6</sup> indicated that the median septum (tissue No. 7) is considered by most authors as the most important, but that his own observations led him to conclude that the less elastic lateral suspensory sheets (tissues No. 4 and No. 5) are the chief means of support and that the median septum merely holds the udder in close proximity to the posterior (rear) abdominal wall. In this connection he explained that the greater elasticity of the sheets comprising the median septum permits a lowering of the udder and an outward protrusion of the teats when the udder is distended with milk. He attached little importance to the fascia or areolar tissue (tissues No. 2 and No. 3) and indicated that the chief functions of the skin are to assist in the support of the fore part of the udder and to prevent undue pendulum movement.

An incision was made through the skin and subcutaneous tissue along the right flank and forward on the abdominal wall to a point somewhat anterior to the front attachment of the udder. In this manner an area of skin was dropped down exposing about half of the lateral surface of the udder and some of the abdominal wall. This showed a layer of fine areolar tissue (tissue No. 2) by means of which the udder is loosely attached to the skin. It is by means of such tissue that the skin assists in supporting and stabilizing the udder. Figure 4, A, shows the location and the appearance of the fine areolar tissue along the line *a— $\alpha$* , where the skin is partly separated

<sup>6</sup> See reference cited in footnote 4.



from the underlying tissues. Areolar tissue of this type supposedly provides also the chief means of attachment between the hide in other parts of the body and the underlying structures. It is obvious from the well-known flexibility of the skin that this does not give a rigid attachment but permits considerable laxity and motility.

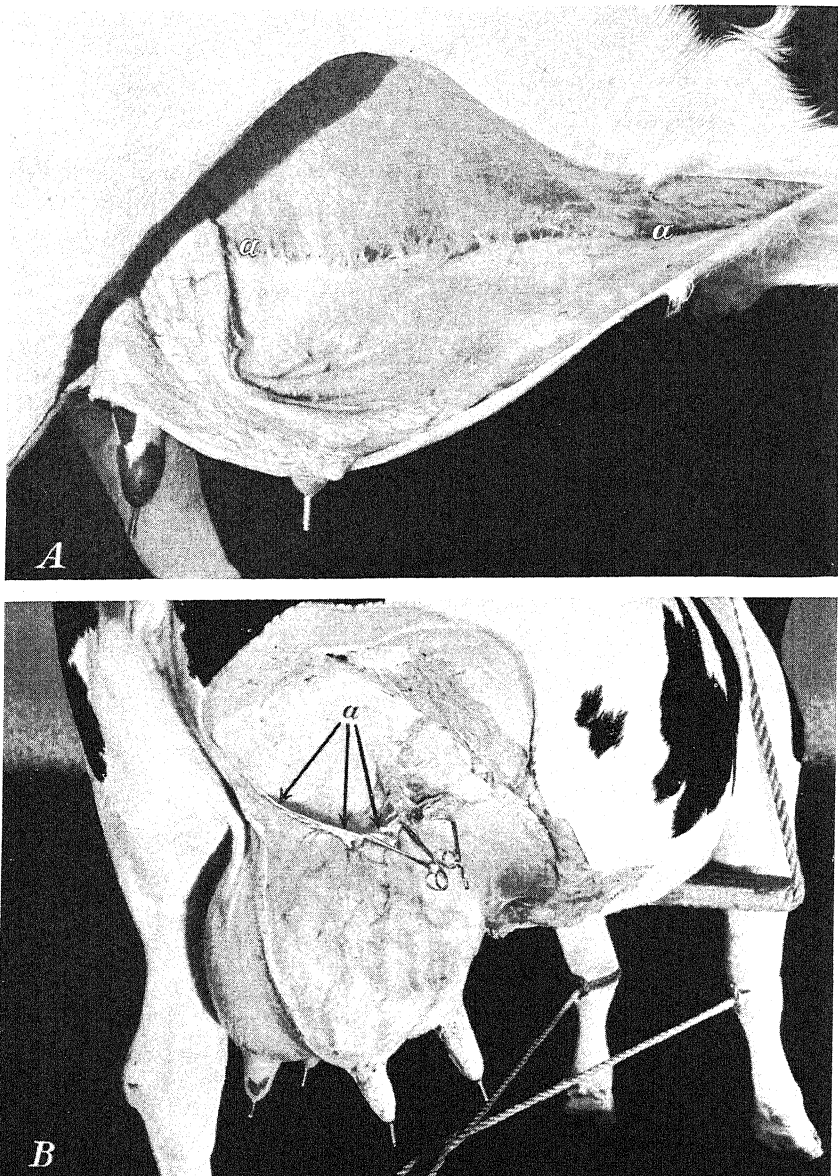


FIGURE 4.—*A*, Location and appearance of the subcutaneous fine areolar tissue (tissue No. 2) where the skin is folded down along the line *a-a*; *B*, portion of a partly enveloping sheet of fibrous tissue (tissue No. 4) which originally had been attached to the muscular tissues of the thigh. The severed edge is shown along the line indicated by *a*. Right hind leg removed.



The right hind leg was next removed at a point just below the hip joint, together with an area of skin extending from the tips of the teats upward to the level of the hip joint (thurl) and forward to a point somewhat anterior to the front attachment of the udder. This revealed more fine areolar tissue (tissue No. 2) and a sheet of fibrous partly elastic tissue which was still attached to the abdominal wall and which originally had been attached to the muscular tissues of the thigh. A portion of this sheet of tissue (tissue No. 4) was severed along the posterior dorsal (rear upper) surface of the udder, and is shown stretched out laterally by means of forceps attached to a cord

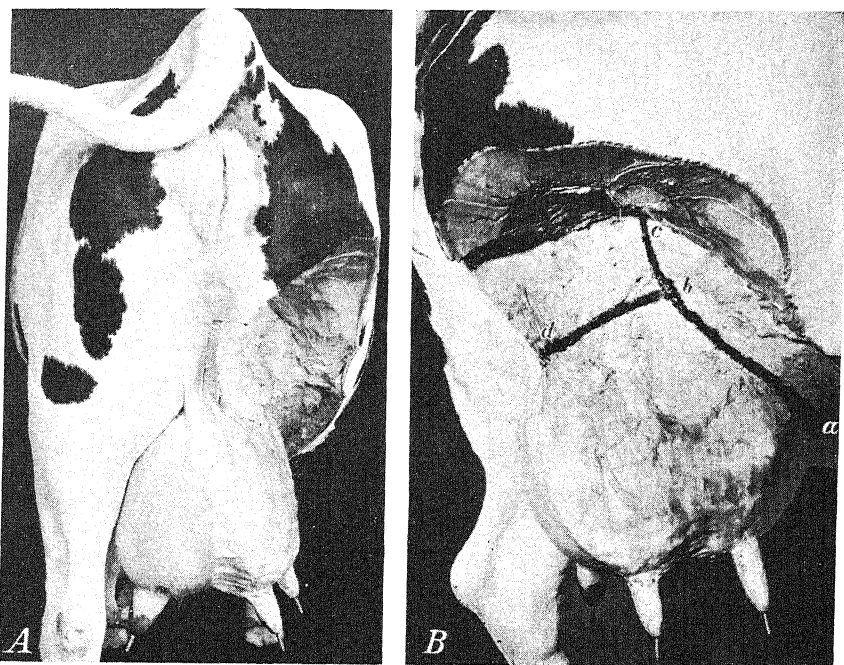


FIGURE 5.—*A*, Posterior view of the udder showing how it narrows dorsally to accommodate the thigh; *B*, lateral view with the upper boundary of the gland tissue outlined with a stain. Line *a—b—c* shows the line of the abdominal wall; *b—d*, the upper boundary of the udder. Note how the upper boundary of the udder follows the contour of the abdominal wall to a point approximately below the brim of the pelvis and then inclines downward toward the rear.

(fig. 4, *B*). It will be noted that this layer of tissue is fairly close to the median line at the rear and that it flares out toward the front. It partly envelops the udder and serves as an important unit of its suspensory apparatus. The line on which the separation was made is indicated by *a*.

A posterior (rear) view of the udder at the same stage of dissection (fig. 5, *A*) shows the contour of the udder and indicates how markedly it narrows toward its dorsal (upper) extremity, especially at the rear, to provide ample space for the thigh. A lateral (side) view, also at the same stage of dissection, shows the approximate upper boundary of the gland tissue which has been outlined with a staining material (fig. 5, *B*). It will be noted that the upper boundary follows the

contour of the abdominal wall to a point almost directly below the brim of the pelvis, which is very close to a vertical plane passing through the two hip joints, after which, in this case at least, it inclines downward toward the rear. It is likely that in cows having a firm rear attachment of the udder, the upper surface of the gland tissue posterior to the brim of the pelvis may continue in a more nearly horizontal plane.

Figure 6, *A*, shows a posterior view of the udder after the removal of both hind legs. The posterior median support consisting of tissues

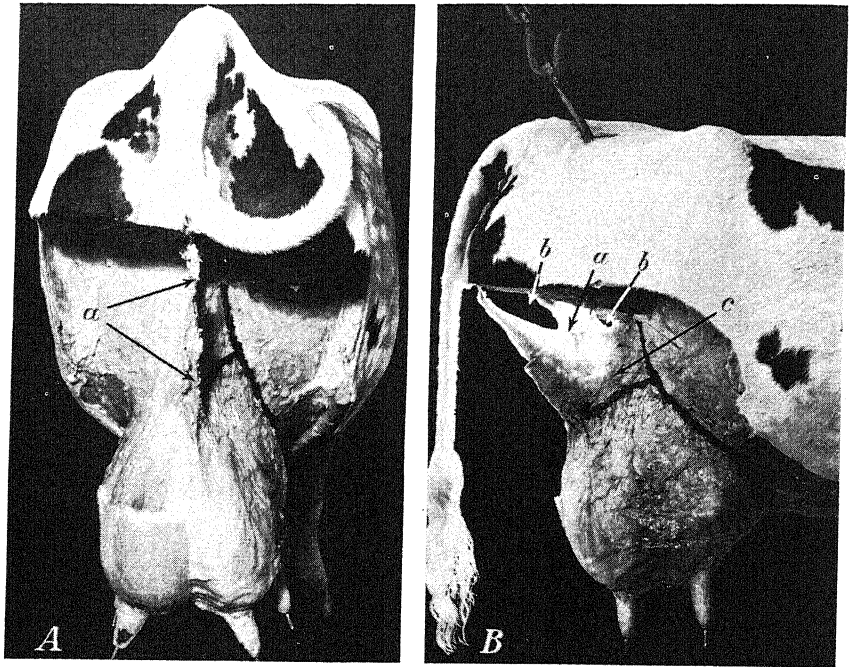


FIGURE 6.—*A*, Posterior view of the udder showing posterior median support at *a*, and indicating the contour of the udder, the angle of abdominal inclination, and the relatively small portion of the abdominal wall covered by the udder; *B*, lateral view showing the translucent subpelvic tendon (*a*) (tissue No. 6) which connects the lateral sheets of connective tissue (tissues No. 4 and No. 5) that are attached to the udder at *c* with the ventral surface of the pelvis. Open spaces in the subpelvic tendon are shown at *b*.

attached to the subpelvic tendon (tissue No. 6) is indicated by *a*. This view also gives a good idea of the contour of the udder itself, the angle of abdominal inclination, and the relatively small portion of the abdominal wall covered by the udder.

A better idea of the nature of the posterior median support of the udder is obtainable from the illustration in figure 6, *B*. This side view shows a closer dissection of the strong subpelvic tendon (tissue No. 6), which connects the lateral sheets of connective tissue that are directly attached to the udder (tissues No. 4 and No. 5) with the ventral (lower) surface of the pelvis (symphysis pelvis). This view, which was illuminated from the opposite side when photographed, shows how the subpelvic tendon becomes increasingly translucent (semi-

transparent) as it changes in structure from the mixture of fibrous and tendinous tissues at the udder, to a thin tendinous sheet where it attaches to the pelvic bone. The subpelvic tendon is not continuous but consists of a number of separated points of attachment along the ventral (lower) ridge of the pelvis. In figure 6, *B*, *a* indicates the translucent subpelvic tendon (tissue No. 6), *b* and *b* indicate the interstices (open spaces) in the tendon, and *c* shows the point where the lateral sheets of the udder's suspensory apparatus (tissues No. 4 and No. 5) arise from the subpelvic tendon.

Despite the inferior rear attachment of this udder its position was not so far forward as to appear unusual in the living cow. However, when the dissection had progressed to the stage illustrated in figure 6, *B*, the udder seemed to be located surprisingly far to the front although actually its position was essentially unchanged.

For purposes of record a number of measurements were made to establish more definitely the size of the udder, the extent of its attachment to the abdominal wall, and its position with reference to other points in the pelvic region. The following are some of the measurements obtained:

	Measurement	Centimeters (Inches)
(1)	Distance from a perpendicular through the posterior point of the pinbone (ischium) to a vertical, transverse plane through the—	
	(a) Posterior (rear) extremity of udder (right side)-----	22.0 ( 8.66)
	(b) Most posterior (rear) extremity of abdominal wall (its junction with the brim of the pelvis)-----	38.5 (15.16)
	(c) Anterior (front) extremity of udder (right side)-----	61.0 (24.02)
	(d) Calculated length of udder-----	39.0 (15.35)
(2)	Greatest width of udder at a vertical plane through rear teats--	29.5 (11.61)
(3)	Greatest width of udder at a vertical plane through front teats-----	32.75 (12.89)
(4)	Maximum width of attachment to the abdominal wall-----	24.75 ( 9.74)
(5)	Width of abdominal attachment at a vertical plane through front teats-----	20.75 ( 8.17)
(6)	Width of abdominal attachment at a vertical plane through rear teats. (See dorsal boundary line in fig. 7, <i>B</i> )-----	8.50 ( 3.35)

Figure 7, *A*, shows a view in which the deep lateral layer of the suspensory apparatus (tissue No. 5) has been partly separated from the abdominal wall to show a layer of cordlike tissue lying between the dorsal (upper) surface of the udder and the abdominal wall. This tissue, which apparently provides the means by which most of the dorsal surface of the udder is attached to the abdominal wall, is areolar in type, very coarse, and loosely bound together. Presumably, in the event of excessive udder weight, or as a result of an inherited weakness or other causes, this tissue might give way and permit a separation between the udder and the abdominal wall with a characteristic breaking away of the front attachment of the udder. Emmerson's comment regarding the relative lack of importance of these tissues is worthy of note. Figure 7, *A*, *a* shows the loose cordlike tissue (tissue No. 3) and *b* the partly severed deep lateral layer of the enveloping suspensory tissue (tissue No. 5).

The completely severed deep lateral layer of the suspensory tissues which attaches to the convex lateral surface of the udder and virtually envelops it (tissue No. 5) is shown in figure 7, *B*. A portion of this layer below the horizontal incision was dissected away from the udder and folded downward along the line *b—b* (fig. 7, *B*) in such

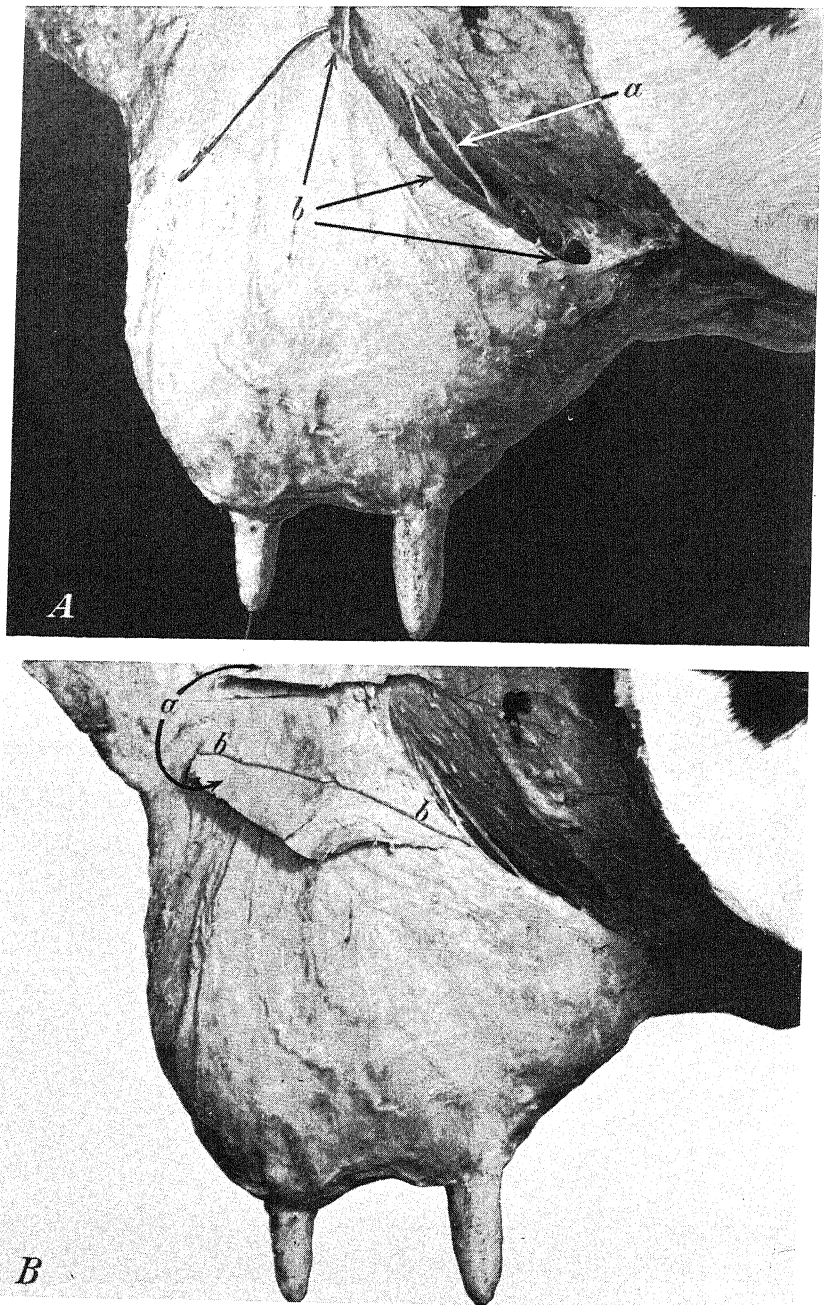


FIGURE 7.—A, The deep lateral layer of enveloping suspensory tissue (tissue No. 5) partly separated from the abdominal wall at *b* to show cordlike tissue (*a*) (tissue No. 3) which serves to hold the udder in contact with the abdominal wall; B, the deep lateral layer (tissue No. 5) severed at *a* and folded back along the line *b—b*.



a manner as to reveal the two edges, indicated by *a*, which were formed by the incision. As shown in the illustration, this layer consisted of a clearly defined sheet of tough tissue which became continuous, ventrally (below), with the udder. The cordlike tissue along the abdominal wall (tissue No. 3) is shown here also, but less distinctly than in figure 7, *A*. It is conceivable that, in cases where the loose, cordlike tissue gives way, the enveloping layer of tissue and the median elastic support (tissue No. 7) may stretch sufficiently to permit a lowering of the udder—especially in front—which would thereby become pendulous.

The deep lateral layer (tissue No. 5) which previously had been severed (fig. 7, *B*) was separated from the udder to a lower level and folded downward along the line *a-a*, shown in figure 8, *A*. Figure 8, *A*, also shows how the dissection on the right side was carried deeper with the removal of a considerable quantity of fat and connective tissue, bringing to light the small areas of parenchymatous (secretory) tissue shown at *b*; the branch of one of the two yellow elastic supporting sheets of tissue (tissue No. 7) shown at *c*, which form the median septum of the udder; one of the large arteries where it enters the udder as shown at *d*; and a large vein on the dorsal (upper) surface of the udder at the point where it emerges from the udder as shown at *e*. The subpelvic tendon (tissue No. 6) is still attached (fig. 8, *A*, *f*).

Figure 8, *B*, shows the udder from the left at essentially the same stage of dissection as in figure 8, *A*, except that more of the deep, lateral enveloping layer of suspensory tissue (tissue No. 5) has been removed from the anterior (front) part of the udder, whereas at the rear of the udder a section of it (fig. 8, *B*, *a*) is still intact and attached both to the mammary gland and to the subpelvic tendon. A large vein extending along the abdominal wall at the upper surface of the udder is clearly visible (fig. 8, *B*, *b*).

It seems appropriate at this point to give attention to a matter that was not satisfactorily brought out in the gross dissection of the udder used in this study. Sisson (7), Emmerson,<sup>7</sup> Turner,<sup>8</sup> and Espe (3, pp. 9-10) mentioned the lamellae (plates) that are given off by the deep faces of the deep lateral sheets (tissue No. 5) which penetrate the udder and join with its intraglandular interstitial framework. A histological study of sections including the lateral sheets and the glandular tissues immediately beneath shows the manner in which the lamellae branch off and extend downward and inward to become continuous with the intraglandular tissues (pl. 1, *A*). This study brought out the fact that the sheets comprising the median septum (tissue No. 7) also give rise to lamellae which penetrate the glandular tissue in a similar manner (pl. 1, *B*).<sup>9</sup>

It is more difficult to determine to what extent the lateral and median sheets fuse at the ventral (lower) border of the gland to form a sling or cradlelike structure for its support. This is because of the laminated nature of both the lateral and median sheets and the fact that numerous branches are given off. A section through an udder that was included in routine studies of the Bureau of Dairy Industry shows a band of tissue that appears to extend continuously around

<sup>7</sup> See reference cited in footnote 4.

<sup>8</sup> See reference cited in footnote 5.

<sup>9</sup> The illustrations shown in plate 1, *A* and *B*, were made available through the cooperation of John D. Hunt, Division of Nutrition and Physiology, Bureau of Dairy Industry.

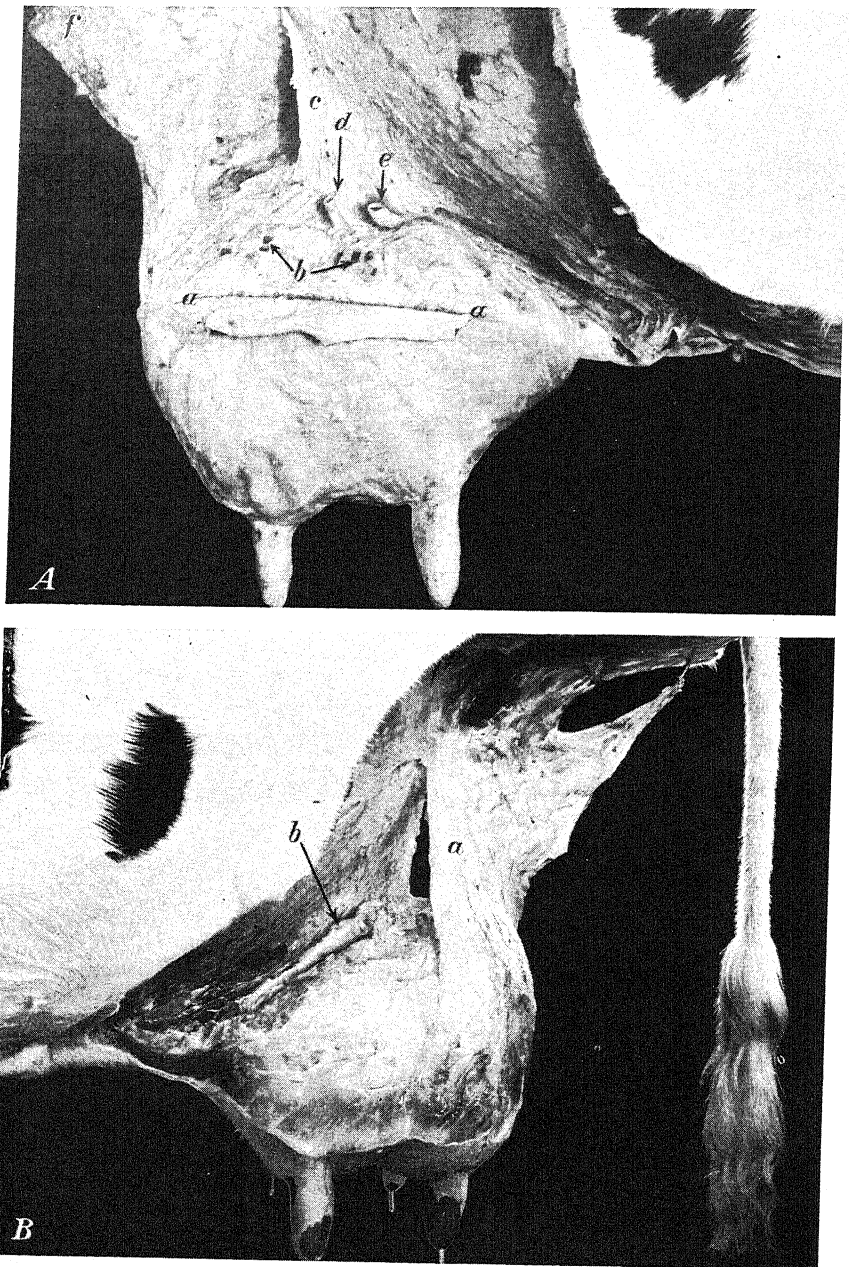
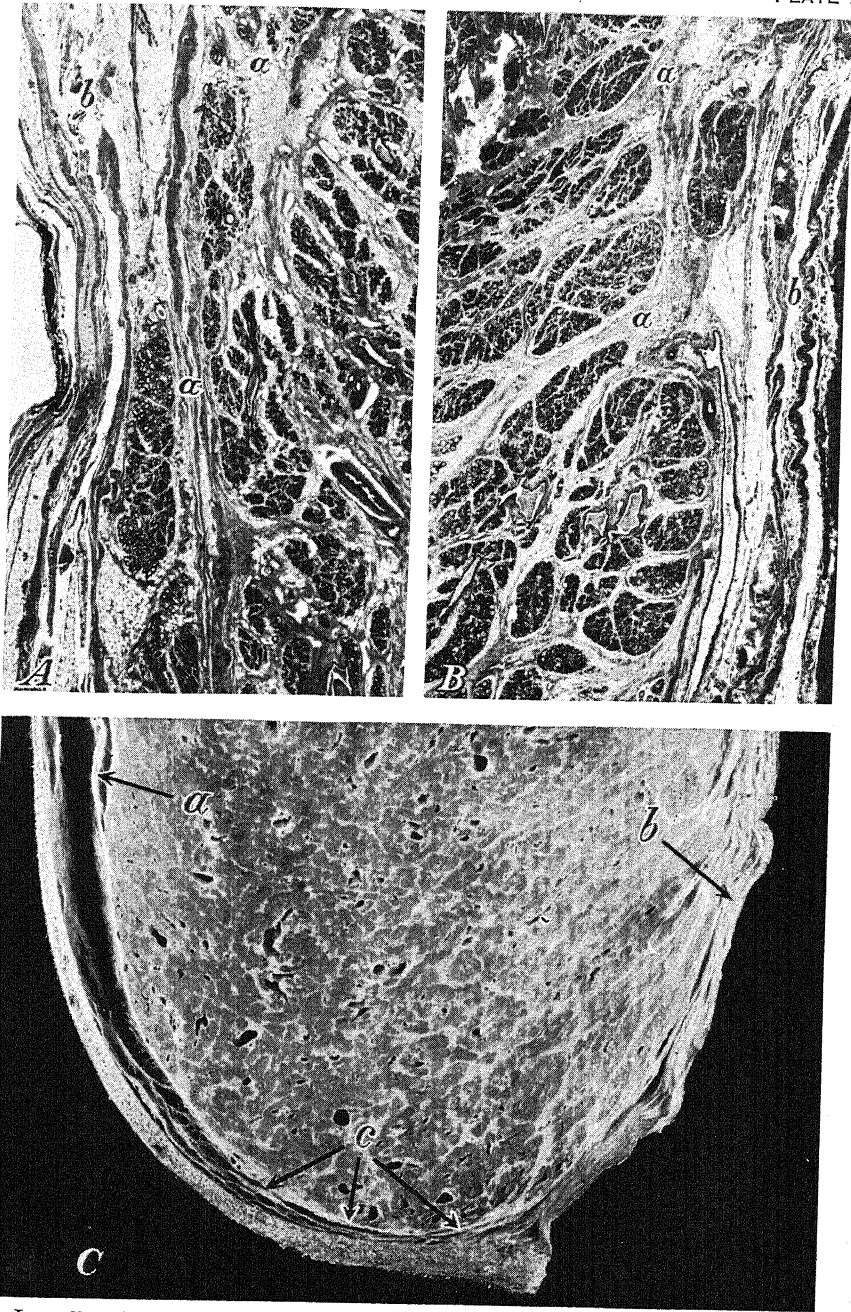
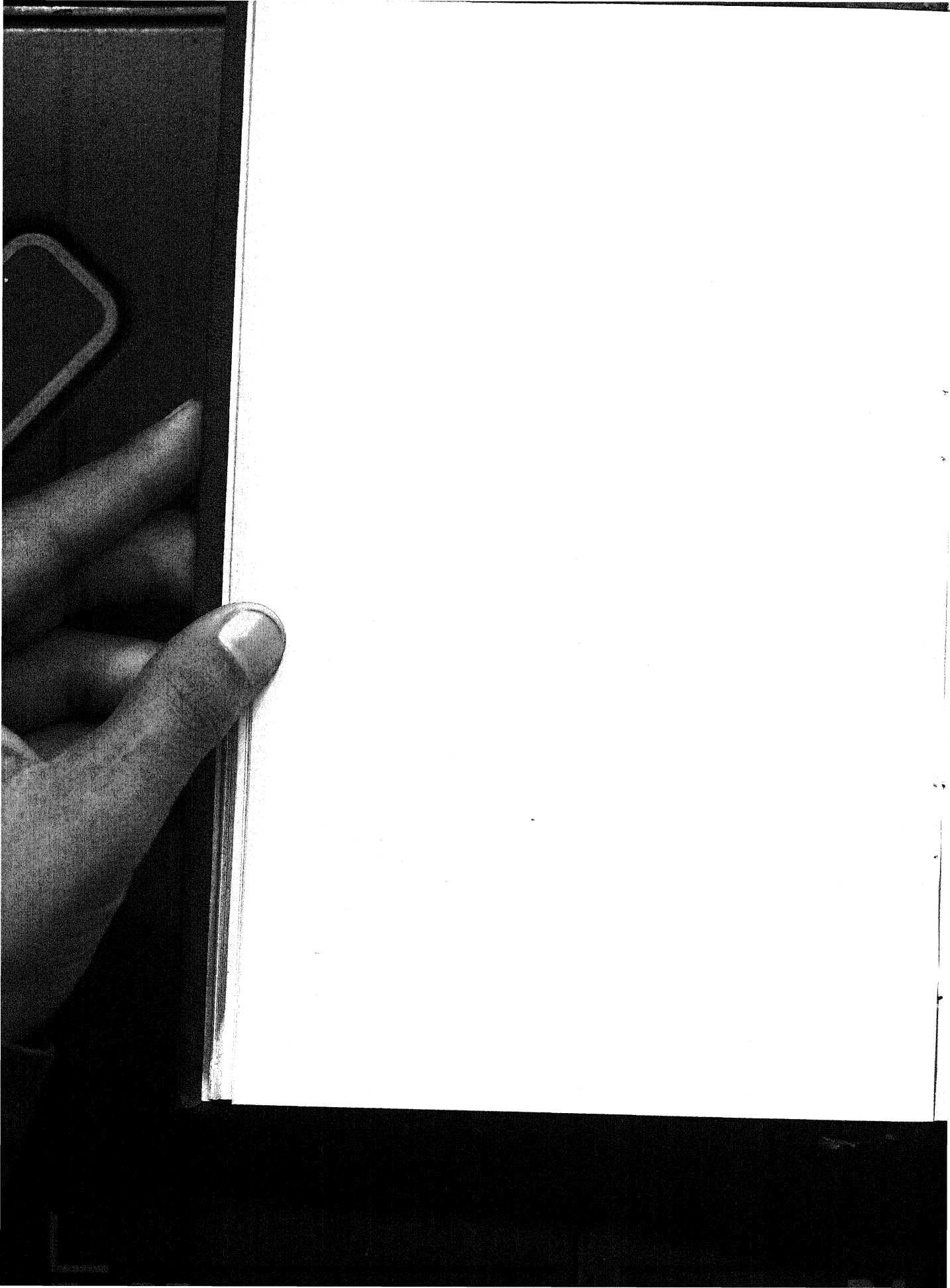


FIGURE 8.—A, Deeper dissection in which the deep lateral layer (tissue No. 5) is folded downward to a lower level revealing small areas of mammary-gland tissue (b), a portion of one of the median yellow elastic supporting sheets (c) (tissue No. 7), a large artery (d), and a large vein (e); the subpelvic tendon (f) (tissue No. 6) is still in position. B, Left side of udder with anterior part of deep lateral layer removed but with posterior part (a) still attached to udder and subpelvic tendon. A large vein is shown at b.



A, Lamellae (*a-a*) branching off from the deep lateral sheet (*b*) (tissue No. 5), and extending inward and downward to become continuous with the intraglandular tissues of the udder; B, similar lamellae (*a-a*) arising from one of the sheets (*b*) (tissue No. 7) which form the median septum; C, a band of tissue that appears to make the lateral (*a*) and median (*b*) layers (tissues No. 5 and No. 7) continuous around the ventral (lower) border of the gland (*c*).





the ventral (lower) border of the gland from the lateral to the median surface (pl. 1, *C*).

In figure 9, *A*, the udder is shown with all of the supporting tissues removed except a small area of areolar tissue and skin at the anterior (front) extremity of the mammary-gland tissue *b*, and the main portion of the deep medial, yellow elastic tissue *a* (tissue No. 7) which is attached to the abdominal wall above and continues below to form the septum between the halves of the udder. Apparently the areolar tissue and skin carried little weight. The white strip *c* between the abdominal wall and the dorsal surface of the udder is the result of light coming from the opposite side. It shows that all the supporting tissues in this area had been completely severed.

In figure 9, *B*, the udder is shown with all supporting structures removed except the main portion of the deep medial yellow elastic tissue *a* (tissue No. 7) that was pointed out in figure 9, *A*. The length (front to rear) of this layer of tissue, as shown, was 16.5 cm. (6.50 inches) where it attached to the abdominal wall, but was only 11.0 cm. (4.33 inches) at its shortest point. The great strength and the nearly perfect location of this median support are noteworthy. The weight of the udder, dissected as shown in figure 9, *B*, with the formalin it contained, was 47.0 pounds. Although the left half of the udder apparently was heavier than the right—a condition that caused the udder to list slightly to one side—its position when suspended from the short narrow sheet of median tissue as shown in figure 9, *B*, was otherwise not significantly changed from its position on the living cow. Measurements of the height of the tips of the rear and front teats were made from time to time as the dissection progressed. The heights measured at midafternoon on January 30, at the conclusion of the dissection shown in figure 6, *B*, were 37.5 cm. (14.76 inches) for the rear teats and 40.3 cm. (15.87 inches) for the front teats. Up to this time no settling or significant change in the position of the udder was apparent. After overnight suspension following the dissection shown in figure 6, *B*, the heights were 37.2 cm. (14.65 inches) and 39.7 cm. (15.63 inches), respectively, for rear and front teats. With the udder supported as shown in figure 9, *A*, the corresponding heights were 36.6 cm. (14.41 inches) and 39.5 cm. (15.55 inches) respectively, and after all of the supporting structures except the main portion of the medial yellow elastic septum had been severed (fig. 9, *B*) the heights were 36.3 cm. (14.29 inches) and 39.5 cm. (15.55 inches) for rear and front teats. These measurements indicate a total settling of only 1.2 cm. (0.47 inch) for the rear of the udder and only 0.8 cm. (0.31 inch) for the front since midafternoon of the previous day during which time all except one of the main supports of the udder had been removed. Obviously the median elastic tissue not only possessed great tensile strength, but it was so nearly perfectly located above the anteroposterior center of gravity that the udder was in almost a perfectly balanced suspension, when all other structures had been removed.

The results observed in this experiment appear to indicate that the medial septum is the principal structure by which the udder is sus-

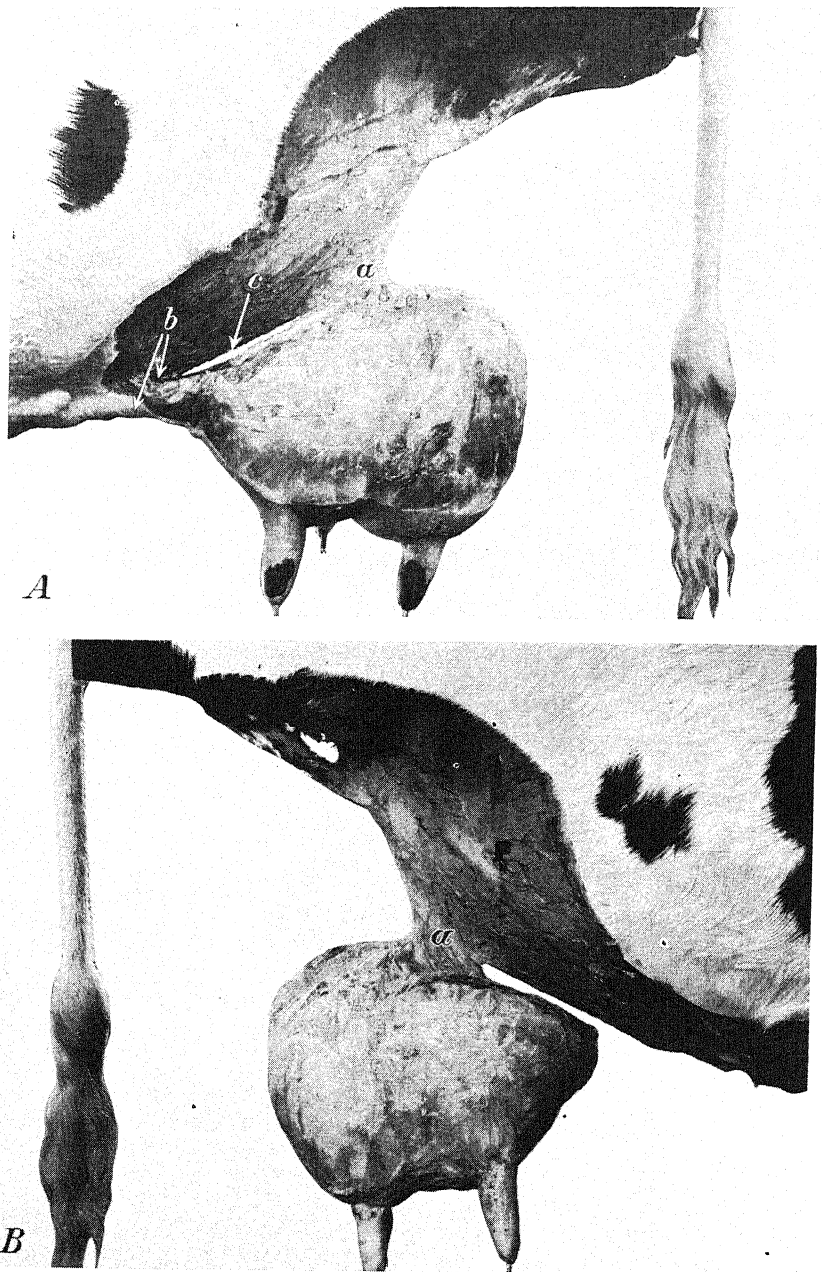


FIGURE 9.—*A*, Udder as seen from left, with all supporting structures removed except the main part of the medial yellow elastic tissue (*a*) (tissue No. 7) and a small area of skin and areolar tissue (*b*); a streak of light shows between the abdominal wall and the udder at *c*. *B*, View from the right with the medial yellow elastic tissue (*a*) remaining as the only support for the udder, note the almost perfectly balanced suspension of the udder.

pendent. No other experiments were conducted to determine to what extent the other membranes could have supported the udder in position. It is of interest to note that the medial septum was attached to the abdominal wall but apparently not directly to any bony structure.

A rear and somewhat superior view of the udder at the same stage of dissection (fig. 10, *A*) indicates the narrowness of the main portion of the double sheet of medial tissue (tissue No. 7) shown in figure 9, *B*, which furnishes one of the chief supports of the udder and forms the septum that separates the two halves. The somewhat wider and heavier left half of the udder and the resulting swing of the entire organ to the right are clearly indicated.

At this point the right half of the udder was removed by means of a longitudinal incision that was carried as close as possible to the right of the median septum. Figure 10, *B*, shows the surface from which the right half of the udder was removed. It indicates the manner in which the supporting medial tissue (tissue No. 7) *a* spreads out in a fan-shaped manner to form a septum that reaches and attaches to nearly all parts of the medial flat surface of the gland. Apparently the fan-shaped distribution of this septum provides maximum support to the udder and accounts for its almost perfectly balanced suspension.

Figure 11, *A*, is a rear view that shows the shape and the appearance of the abdominal wall after the medial support of the udder was severed along the line *a—b*. In this view *b* shows a severed abdominal vein ("milk vein"), *c* is one of the ventral (lower) points of the symphysis pelvis to which the subpelvic tendinous support of the udder was attached, and *d* and *d'* are the front legs of the cow.

Figure 11, *B*, is a lateral view of the abdominal wall at the same stage of dissection. It shows both the angle of inclination and the degree of curvature of the abdominal wall from the point at *b*, where the anterior (front) extremity of the udder had been attached, to the pubis at *c* (anterior part of pelvic floor). In this view a point of the symphysis pelvis (under median surface of pelvis) is shown at *d*, and the tendinous stump which formed the point of attachment for the median support of the udder is again indicated by *a*.

In addition to the photographs shown in figure 11 a number of measurements were taken to show the shape and position of the abdominal wall in the area where the udder had been attached. This study of the shape of the abdominal wall in relation to that of the dorsal (upper) surface of the udder was undertaken for the purpose of providing, if possible, some means of estimating the hidden boundaries of the udder and thereby of calculating udder volume in the living animal. The measurements were made with the embalmed cadaver still supported in the position shown in figure 2, *A*, with the udder and extraneous tissues dissected away as shown in figure 11. The measurements consisted of (1) horizontal distances from a perpendicular transverse plane located at the posterior extremity of the pinbone (ischium) to specified, previously located points; and (2) heights of the same points above the surface (of the stoneboat) to

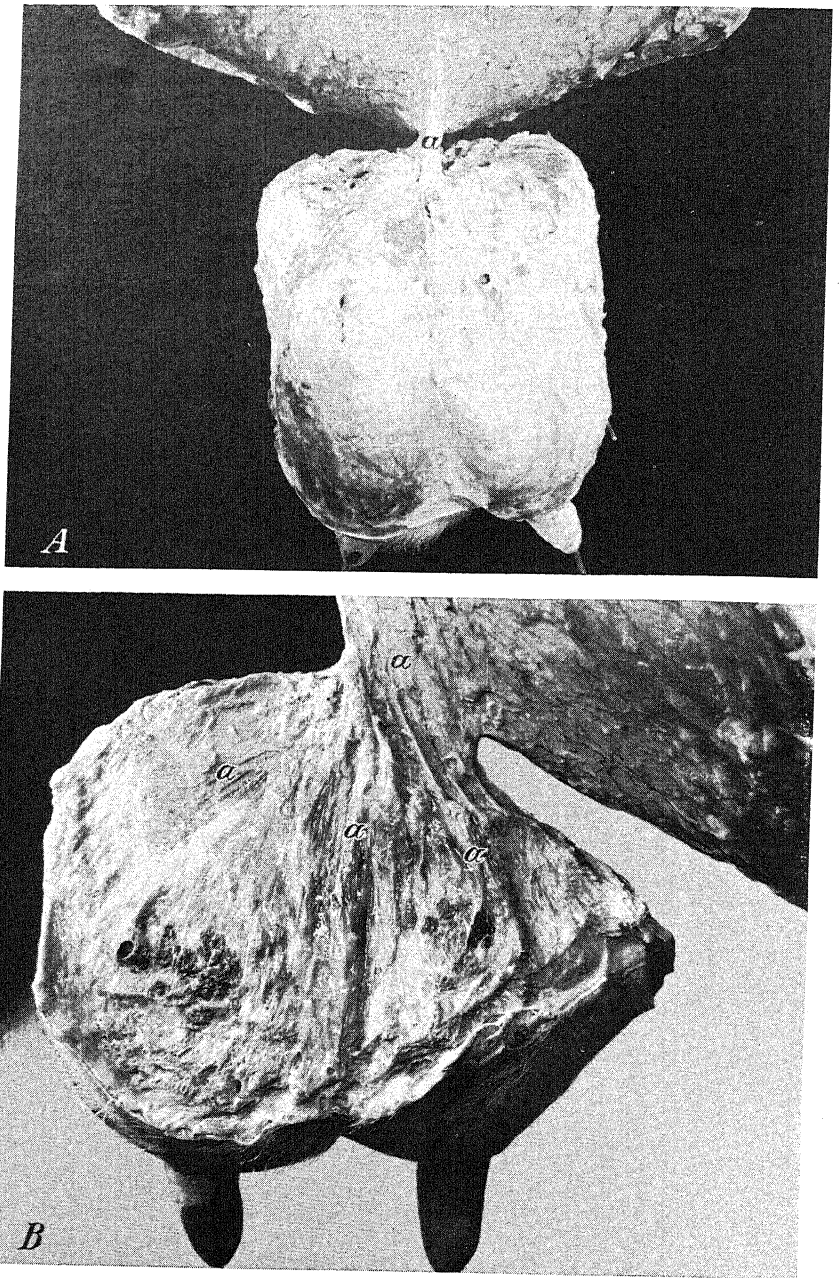


FIGURE 10.—*A*, Rear view of the udder from slightly above, showing the narrowness of the main part of the medial yellow elastic tissue (*a*) which is capable in itself of supporting the udder without material change of position; *B*, surface from which the right half of the udder has been separated to illustrate the fan-shaped attachment (*a*) of the septum to the medial surface of the udder.



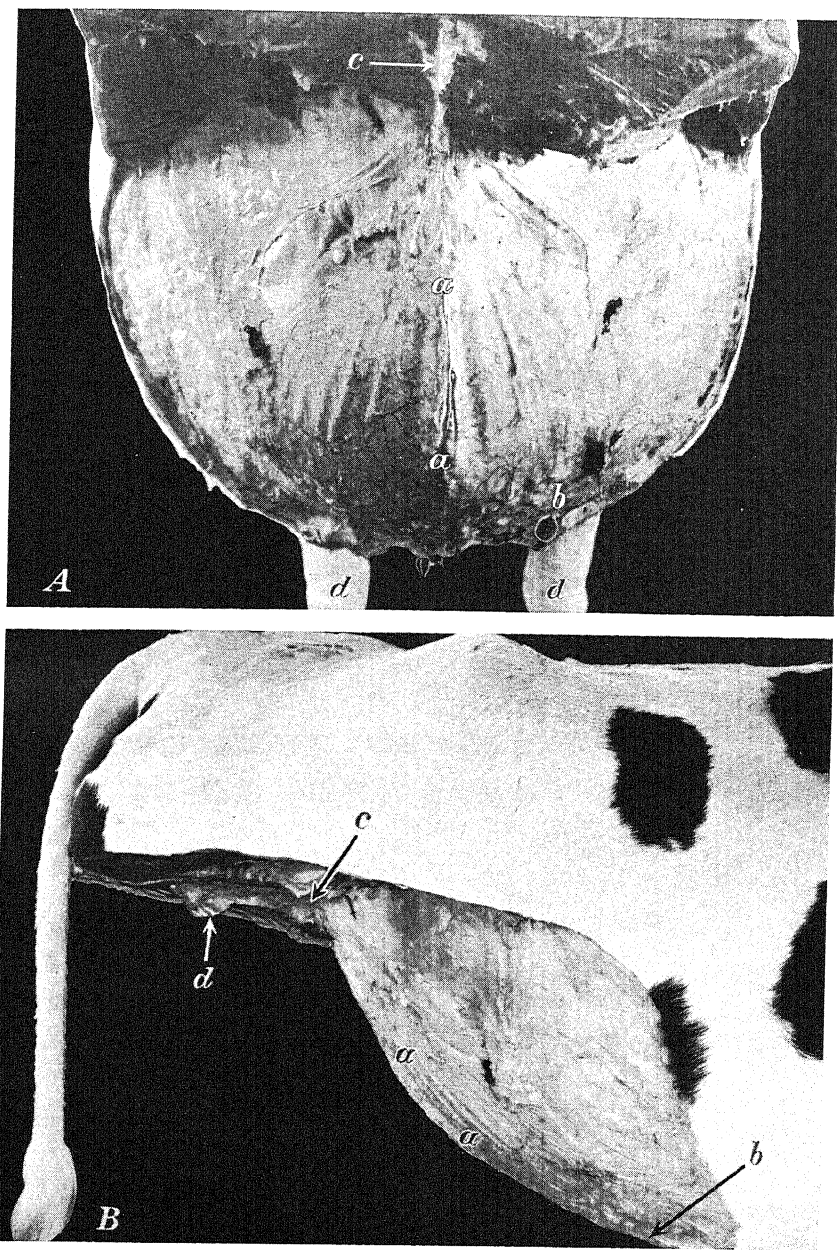


FIGURE 11.—*A*, Rear view of the abdominal wall after removal of udder: *a*—*a*, The line where medial septum was severed; *b*, a milk vein; *c*, one of the points at which the subpelvic tendon was attached to the pelvis; *d*, the front legs. *B*, Lateral view of the abdominal wall: *b*, The anterior point of attachment of the udder; *c*, the approximate location of the brim of the pelvis; *d*, the symphysis pelvis; and *a*—*a*, the tendinous stump of the medial yellow elastic supporting septum. Note that this medial septum was attached to the abdominal wall and not directly to any bony structure.

which the cow's feet were attached. The measurements obtained were as follows:

	Measurement	Centimeters	(Inches)
(1)	Anteroposterior length on the median line from a vertical transverse plane at the posterior extremity of the pinbone (ischium) to the intersection of the abdominal wall and a vertical transverse plane through—		
	The navel.....	88.50	(34.84)
	The anterior attachment of the udder.....	62.00	(24.41)
	A front teat (right).....	49.50	(19.49)
	A rear teat (right).....	35.75	(14.07)
(2)	Height of the abdominal wall at the median line, above the surface to which the feet had been attached (stoneboat), in a vertical transverse plane through—		
	The navel.....	56.00	(22.05)
	The anterior attachment of the udder.....	62.75	(24.70)
	A front teat (right).....	73.75	(29.04)
	A rear teat (right).....	92.00	(36.22)

From these measurements the medial anteroposterior (front to rear) curvature of the abdominal wall was plotted. The plotted curve is shown in Figure 12, *A*, in which the line *o—x* represents the vertical transverse plane at the posterior (rear) extremity of the pinbone; *o—y*, the surface to which the cow's feet were attached (stoneboat); *a*, the navel; *b*, the anterior attachment of the udder; *c*, a vertical transverse plane through the front teats; and *d*, a vertical transverse plane through the rear teats. The distances from *o—x* and *o—y* to the points *a*, *b*, *c*, and *d* are shown in the graph.

By the use of special apparatus three contours were drawn (fig. 12, *B*) to show the transverse curvature of the abdominal wall at the vertical transverse planes passing through the navel (*a*), the anterior attachment of the udder (*b*), and a front teat (*c*). Plane *d* intersected the abdominal wall at a point too close to its junction with the pelvis to permit making a significant contour.

#### SUMMARY AND CONCLUSIONS

This study has made it possible to visualize and to illustrate the position and appearance of the principal structures by which the cow's udder is suspended. The fine areolar subcutaneous tissue by means of which the skin covering the udder is attached to the underlying tissues; the cordlike coarse areolar tissue which forms a loose connection between the upper surface of the front quarters of the udder and the abdominal wall; the superficial lateral sheets which arise from the subpelvic tendon, extend downward over the udder and attach to the thigh; the deep lateral sheets that have a similar origin but which virtually envelop the udder and attach directly to its outer surfaces by numerous plates that pass into the gland; the subpelvic tendon itself, from which the superficial and deep lateral layers arise; and the heavy yellow elastic sheets which arise from the abdominal wall, form a fan-shaped septum between the two halves of the udder, and serve as its chief median support and stabilizer, are all clearly shown in the illustrations that accompany the discussion.

Fleshy udders, udders that become edematous, and the udders of high-producing cows often become excessively large and heavy—particularly with advance in age—and overtax the structures by which they are suspended from the body. It is not difficult to imagine how, in these very large udders, any or all of the various sheets of supporting

tissues might become lax and stretch, and how the areolar tissue might pull apart in cases where any of the other tissues lose their normal tonus. It is likely also that a weakness in the suspensory

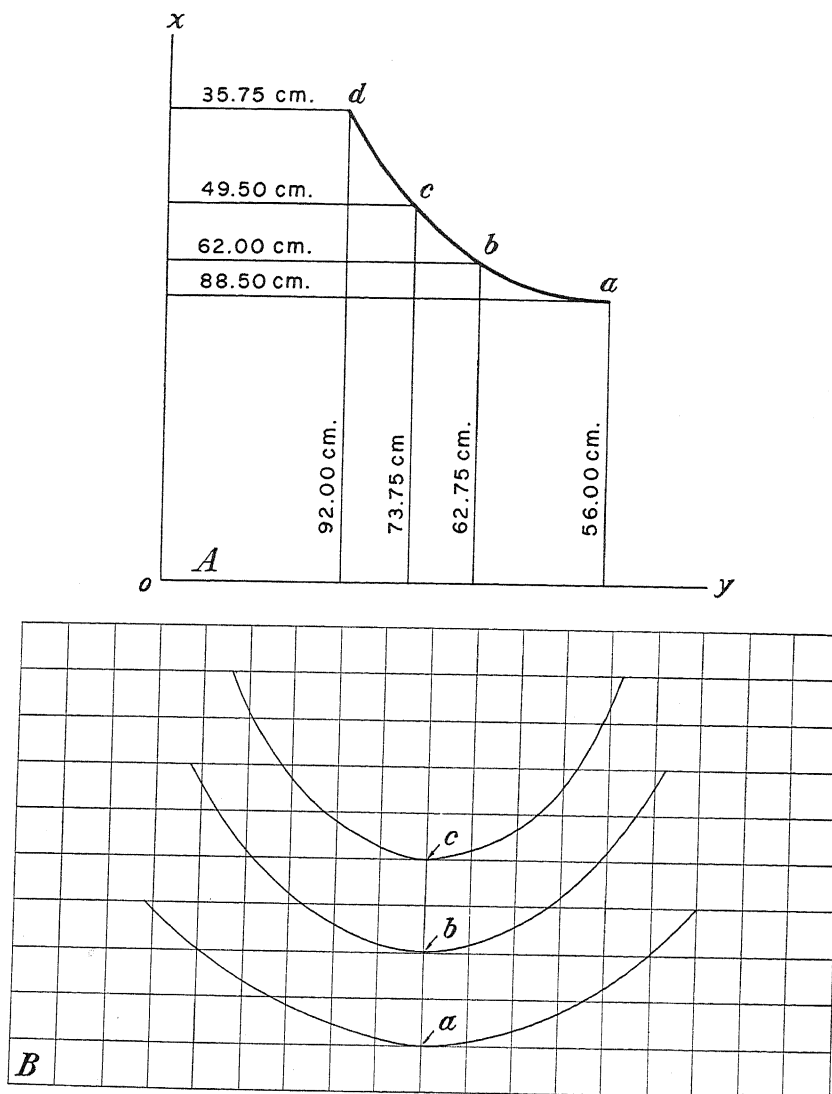


FIGURE 12.—A, Curve constructed from measurements to show the medial anteroposterior (front to rear) curvature of the abdominal wall from the navel ( $a$ ) to the brim of the pelvis ( $d$ ); measurements show distances from the floor and from the perpendicular at the rear of the pinbone (see text). B, The transverse curvature of the abdominal wall:  $a$ , At the navel;  $b$ , at the anterior (front) attachment of the udder; and  $c$ , directly above the front teats.

apparatus might occur as a result of inheritance or of a generally poor physical condition, and bring about a similar "breaking down" in udders that have not become excessively enlarged by fleshiness,

edema, or high production. The occurrence of poorly supported udders in young cows—sometimes early in first lactation—is an indication that such is the case.

The great tensile strength of the median elastic support of the udder (tissue No. 7), and its almost perfect location above its center of gravity are particularly noteworthy. In the case studied the main portion of this median support, which was less than 4½ inches long (front to rear) at one point, and had only a 6½-inch attachment to the abdominal wall, was capable in itself of holding the udder in an almost perfectly balanced suspension. Undoubtedly its fan-shaped attachment to the medial faces of each half of the udder accounted largely for the balance of the udder.

It probably is this median supporting tissue that becomes excessively relaxed in cases where the udder sags along the median line and the teats point outward laterally. It is likely also that a lack of tonus in the lateral sheets of supporting tissue (tissues No. 4 and No. 5), together with a separation of the fibers of the areolar tissues (tissues No. 2 and No. 3), may cause the udder to break down on the sides (front, rear, or both) with the result that the active support becomes very narrow and appears to be almost entirely median.

The dorsal (upper) surface of the udder does not, as has sometimes been supposed, follow a continuous curve from its anterior to its posterior attachments. It appears to follow quite closely the curve of the abdominal wall toward the rear to a point approximately under the brim (front) of the pelvis, but subsequently it carries out in a generally horizontal plane or may incline downward. In the case of the cow studied the dorsal surface of the udder posterior to the brim of the pelvis inclined rather definitely downward toward the rear. In this connection the poor rear attachment of the udder in this animal both as a calf and as a cow is again pointed out.

It is noteworthy that in the cow dissected the junction of the abdominal wall and the pubis—almost directly below the acetabulum (hip joint)—was located superiorly (above) and essentially in a vertical transverse plane passing through the rear teats. Presumably the relative location of the teats with reference to these points will vary with individual cows, depending largely on the shape and position of the udder.

The study of this cow emphasized the fact that the area of contact between the dorsal surface of the udder and the abdominal wall was very much smaller than the area of a horizontal transverse section through the udder.

Owing to the fact that the dorsal (upper) surface of the udder does not attach directly to the abdominal wall, or coincide with it in shape, posterior (to the rear) to a plane approximately at the midpoint of the udder, it is doubtful whether the curvature of the abdominal wall can be used as a reliable guide in estimating the size or volume of the udder in the living cow. The difficulty in making an accurate estimate of udder volume is complicated also by the fact that the dorsal (upper) contour of the udder is dependent to a very great extent on the quality and nature of its suspensory apparatus, which appears to vary greatly in individual animals. Moreover, such an estimate is likely to be inaccurate on account of the possibility that laxity in the abdominal muscles may alter the shape and position of the udder.

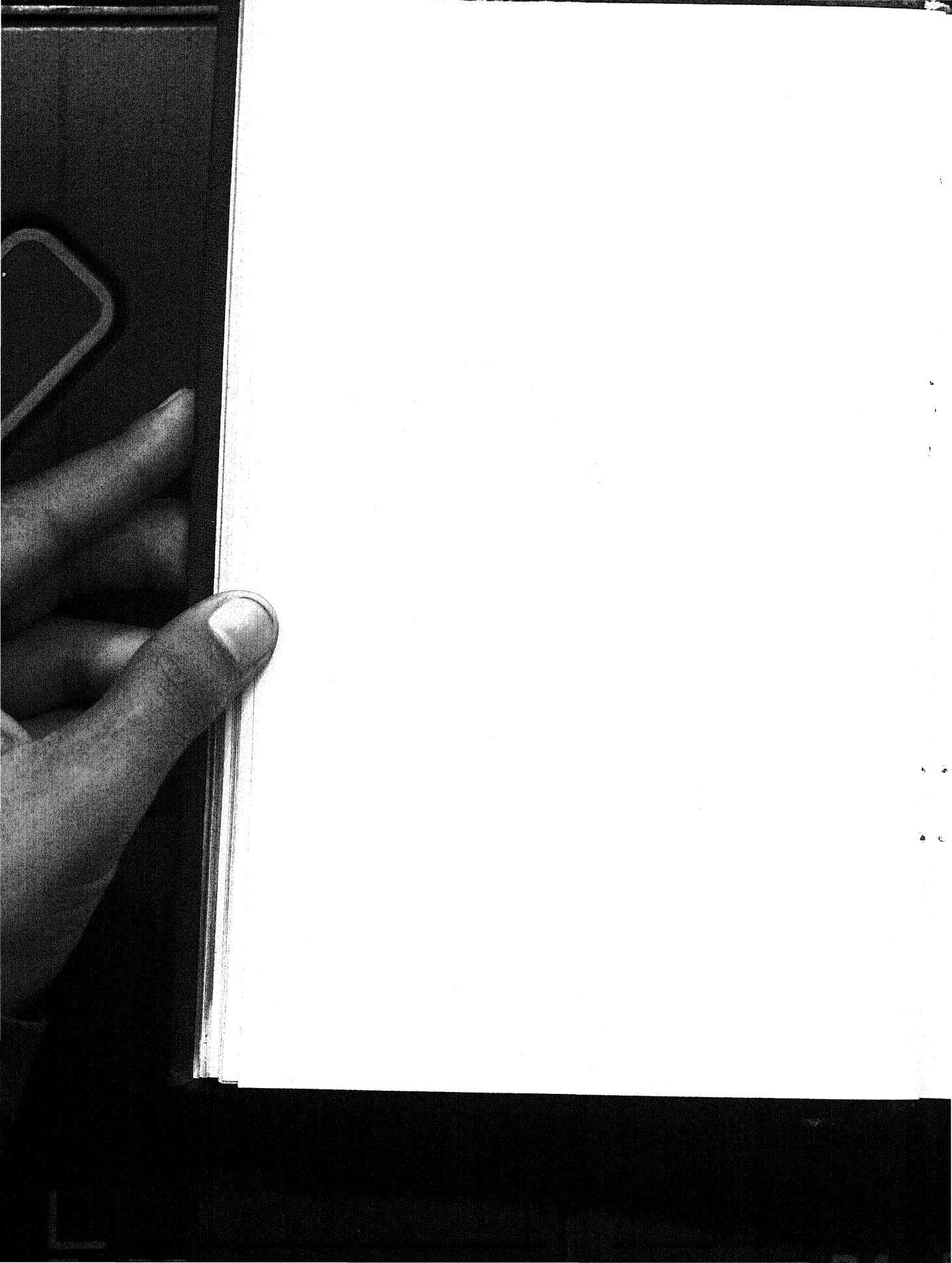
This study, by illustrating the form and nature of the apparatus by



which the udder is attached to the cow's body, should make possible a better understanding of the conditions that may exist and of the anatomical changes that may have taken place, as a result of an udder becoming "broken down" and pendulous. If, as it appears, an inherited weakness in the suspensory tissues, and excessive weight of the udder and its contents are two of the chief factors contributing to the breaking down of the udder, the selection of breeding stock that are known to have an inheritance for well attached udders, and more frequent milking of heavy producing cows, should be effective as preventive and remedial measures.

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# A MICRURGICAL STUDY OF CROWN GALL INFECTION IN TOMATO<sup>1</sup>

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## INTRODUCTION

This paper is concerned with micrurgical studies on crown gall infection in tomato (*Lycopersicon esculentum* Mill.), with emphasis on the relation of inoculation technique to infection. Despite a voluminous literature on crown gall (11),<sup>2</sup> little is known of the quantitative aspect of the infection mechanism, particularly the limits in wound size and in bacterial numbers that govern infection.

## LITERATURE REVIEW

Levine (9) studied the effect of inoculating tobacco plants with various quantities of different dilutions of the crown gall organism (*Phytoplasma tumefaciens* (Sm. and Town.) Bergey et al.). He used suspensions ranging from ordinary agar culture washings to dilutions of 1:100, but he found no marked difference in the size of the galls resulting from any of the preparations. Manifestly, however, even in the 1:100 dilution, the number of bacterial cells must have been in the thousands. The results of this work emphasize the limitations of the dilution method and the indispensability of micrurgical or micromanipulative technique in studies involving small numbers of micro-organisms.

In phytopathology, while the most extensive application of micrurgy has been in connection with the isolation of single cells to obtain pure cultures (4, 6), other applications have involved pathological studies of bacterial, virus, and fungus diseases (1, 5, 7, 8, 10, 12, 13, 14). Hildebrand (5) was the first to demonstrate that a single bacterium is capable of inducing disease in plants.

In animal pathology, although there are reports that as few as one individual tuberculosis germ can produce infection, Webb, Williams, and Barber (17) induced tuberculosis in a guinea pig by subcutaneous inoculation with a minimum of 20 tubercle bacilli. They refer to work by Wyssokowicz in which he reported that 8 tubercle bacilli were able to set up an infection in the peritoneal cavity of the guinea pig and that 24 to 30 bacilli were required in the rabbit. They also report that on 4 different occasions 1 thread (3 to 6 bacilli), isolated directly from the blood of a mouse dying from anthrax, caused death when inoculated into healthy mice.

<sup>1</sup> Received for publication November 1, 1941. The work reported here was begun in 1939 at the Rockefeller Institute for Medical Research, Princeton, N. J., during tenure by the writer of a Guggenheim fellowship. The writer was on sabbatical leave from Cornell University in 1939-40, and the work was later completed at Cornell. All the diagrams were prepared by Mrs. D. W. Thomas.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 58.

Thöni and Thaysen (15), employing between 10 and 343 tubercle bacilli per inoculation dose, were able to get infection in guinea pigs in but 1 instance, with 71 cells. Although the results of these investigators seem to contradict those of earlier work, it should be mentioned that they used Burri's (3) india-ink method, which because of optical difficulties has rarely been used for isolating bacteria, whereas the method of Barber (2), employed with some modifications by Webb, Williams, and Barber (17), remains even today perhaps the most reliable and widely used technique for isolating single micro-organisms (6).

In 1926 Wámoscher (16) conclusively demonstrated for the first time the infectivity of single pneumococcus cells in mice, when 5 out of 21, or 23.8 percent, of the mice that received single cells died from the disease. He obtained a maximum infectivity of 83.3 percent death, with dosages consisting of 11 to 20 bacterial cells per mouse. In making isolations of single cells from blood he used a Peterfi micromanipulator and dark-field condenser. For inoculation the pipette was introduced into a wound in the skin and the tip was broken off underneath the skin surface with a tweezer.

The present paper, an abstract of which has already been published (8), reports the results of micrurgical studies with the crown gall organism and attempts to evaluate the significance of wound size and bacterial population in the infection mechanism.

#### MATERIALS AND METHODS

Bonny Best tomato plants selected for size and uniformity (about 6 inches tall) were used in all experiments.

Several crown gall cultures were used but the chief reliance was placed on the peach strain of N. A. Brown, obtained from Dr. A. C. Braun of the Rockefeller Institute. Highly motile young cultures grown in nutrient media for 9 to 15 hours were ordinarily employed.

The micrurgical apparatus consisted of a double Chambers micromanipulator and accessories (6) in modified arrangement for isolation of the bacteria under one microscope and their immediate transfer to the infection court of a plant under a second microscope. Inoculation was accomplished either by a simple shift of the micromanipulator from one microscope to the other or by removal of the pipette containing the inoculum from the holder and manipulating it in the hand during inoculation.

Single-cell isolation was accomplished with the method described by the writer (6). By this method one or any desired number of young motile cells could be isolated in preparation for transfer to infection courts.

The smallest wounds tested as infection courts were made into individual living cells in the intact tomato plant with a pipette having a tip diameter of about  $3\ \mu$  (figure 1). The basal cells in the trichomes or large hairs and the largest epidermal cells adjoining the basal cells of the trichomes on the petiole or stem surface of the tomato were chiefly employed. India ink and olive oil also were injected into living plant cells to check the value of the technique as a means for introducing materials.

The next larger size of wound tested as an infection court was produced by gently stroking the tomato stems once with the side of a polished dissecting needle previously dipped into a bacterial sus-



pension (figure 3, A). By this means the trichomes as well as the smaller glandular hairs in the rubbed strip were crushed and small wounds produced both on the hairs themselves and where they joined the epidermis underneath. This operation was performed under a binocular dissecting microscope. The injuries produced appeared to be limited to one or a small number of cells at the base of the hairs. Stems stroked but not inoculated later showed superficial light-colored scars where the hairs had been removed, indicating the very limited character of the injuries.

This method of inoculation was chosen only after nine different ways of producing tiny wounds in individual plant cells or in very small numbers of plant cells on the stem surface had been tried. These

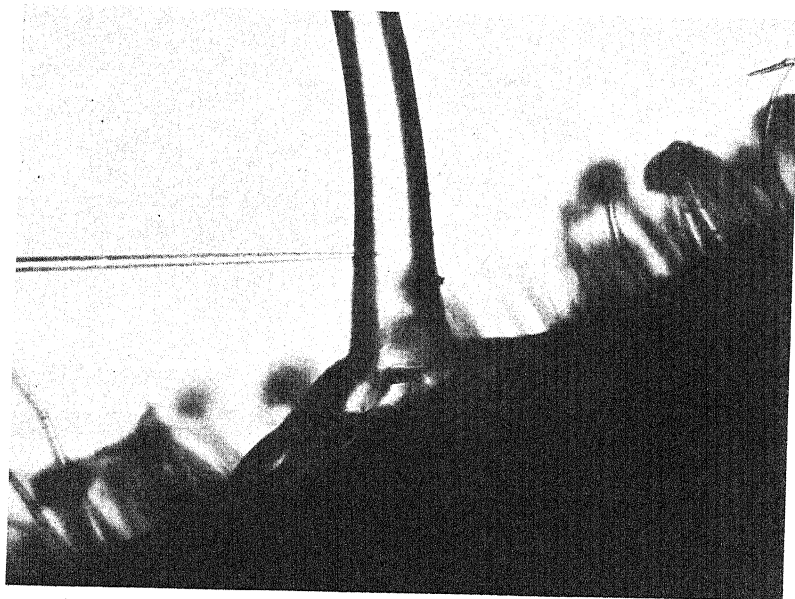


FIGURE 1.—A microinjection pipette inserted into the basal trichome cell on a tomato stem.  $\times 50$ .

methods of wounding, tested on material under the microscope, were as follows: (1) pinching off the tips of trichome hairs approximately 2 cells away from their basal stem attachment with tweezers that had previously been dipped in a bacterial suspension; (2) pinching off trichomes back to 1 cell away from the stem attachment; (3) removal of trichome hairs with a tiny razor so as not to injure the neighboring basal cells; (4) pulling off trichome hairs with square-tipped tweezers, which often resulted in injury to 1 or more of the neighboring cells; (5) gently stroking the tomato stem with the side of a polished dissecting needle; (6) more vigorous stroking of the needle on the stem surface so as to cause collapse of hairs and injury to epidermal cells underneath; (7) making very shallow wounds by puncturing the epidermis with a small dissecting needle to the depth of 2 to 4 cells; (8) using a similar needle for puncturing the epidermis to a depth of 5 to 8 cells; and (9) using a similar needle for puncturing to a depth of 10 cells or more.

In preliminary tests of methods 1 to 6 the wounding instrument was always dipped in a bacterial suspension so that the amount of inoculum applied was not controlled, although the number of bacteria that got into the microscopic wounds was undoubtedly very small.

Wounding large hairs by methods 1, 2, and 3, always gave negative results, which supports the findings of earlier tests in which individual cells of trichome hairs were injected with bacterial suspensions. Occasional tiny galls resulted in from less than 1 to 10 percent of the trials in wound types 4, 5, and 6, with greatest incidence in 6, where most injury was produced.

Wound types 7, 8, and 9 resulted in gall formation in a relatively higher percentage of cases, apparently because these wounds were larger than the minimal size necessary for infection as represented in wound types 4, 5, and 6.

Deep wounds, to depths of approximately one-fourth, one-half, and completely through the stem, produced under the microscope, were tested as infection courts for different amounts of inoculum. The side of the stems receiving such wounds, as well as those of types 7, 8, and 9, were stroked previously with a smooth needle so as to remove the pubescence, an obstruction which interfered with working on shallow needle-puncture wounds. Following the withdrawal of the needle from both the shallow and deep needle-puncture wounds, sterile juice extract was added from a pipette to provide a protruding meniscus to the wound cavity for facilitating the transfer of inoculum. Immediately after inoculation the plants were placed in a moist chamber for approximately 1 hour to retard the drying of the wound and to allow for penetration by the bacteria.

Sterile juice extract for the purpose indicated and for testing as a growth medium was prepared from the foliage of young, rapidly growing plants. The material was ground in a food chopper, extracted through cloth, centrifuged, filtered through a bacteria-proof filter, and then used immediately or stored in a refrigerator until used.

## EXPERIMENTAL RESULTS

### GROWTH MEDIA

#### JUICE EXTRACT FROM TOMATO

Sterile juice extract prepared from the stems and leaves of young tomato plants was found to be an excellent medium for culturing single cells of the crown gall organism. Approximately 90 percent of the single cells grew in microculture when transferred to small droplets of the extract of three different preparations of sterile juice. The isolation technique earlier described by the writer (6) was used. Three such experiments were conducted which involved the transfer of 9, 11, and 10 active young (10 to 15 hours old) single bacterial cells to as many microdroplets, of which 8, 10, and 10 grew.

The fact that single cells of the crown gall organism grew and multiplied readily in the juice extract from tomato plants indicated that individual bacterial cells should be able to grow also in the plant sap contained in wound cavities of the tomato and possibly even within individual plant cells if such an environment should prove to be congenial and a suitable inoculation technique could be developed.

## WOUND SAP

Proof that single cells of the crown gall organism had multiplied in the sap in wound cavities were demonstrated by isolation experiments. Five days after deep wounds had been inoculated with single bacterial cells, and before visible evidence of gall formation, wound tissue was dissected from 20 plants, crushed in distilled water in Petri dishes, and poured with nutrient dextrose agar. Seven days later bacterial colonies like those produced by the crown gall organism were noted on 6 plates. In 1 plate there were about 50 colonies, but in the other 5 the colonies were too numerous to count and were estimated to be in the thousands. Representative colonies were inoculated into tomato plants with positive results, leaving no doubt as to the identity of the organism.

As an outgrowth of these preliminary experiments the author set out to discover, if possible, (1) the smallest size of wound that could be used for infecting tomato plants, (2) the smallest number of crown gall bacteria that would produce infection, and (3) the relative influence of number of bacteria in the inoculum and size of wound on the ultimate size of the galls.

## SMALLEST SIZE OF WOUND REQUIRED FOR INFECTION

## MICROINJECTION INTO INDIVIDUAL PLANT CELLS

One hundred and fifty individual plant cells (120 trichome hair cells and 30 epidermal cells) were inoculated by injection with a bacterial suspension (table 1). The micropipette used for inoculation was introduced into the plant cells under the control of a micromanipulator (fig. 1). When the cell wall was perforated with the pipette tip a hemispherical droplet of protoplasm gushed out and then quickly returned into the cell. At the precise moment that the latter process

TABLE 1.—Summary of infection experiments involving various types of wounds and amounts of inoculum

Kind of wound	Tool used	Bacteria used as inoculum	Experiments	Plant cells or stems inoculated	Crown galls induced			Range in radial extension
					Number	Percentage	Range between experiments	
Microwound ( $3\mu \pm$ ).....	Micropipette.	Number	Number	Number				
		Many	10	150 cells.....	0	10	Percent	Milli-meters
Microwounds (injury to 1 or more epidermal cells).	Polished needle.	Many	10	500 stems.....	Many	25.0	-----	1-2
Shallow wounds (2-4 cells, 5-8 cells, and 10-12 cells deep) grouped <sup>1</sup> .....	{ do.....	1	10	102 stems.....	10	10.0	{ 0-25 6-30 10-36	{ 1-7
		2	9	80 stems.....	8	10.0		
		5	9	72 stems.....	10	14.0		
		10	9	90 stems.....	18	20.0		
		50-100	5	43 stems.....	9	21.0		
Deep wounds (one-fourth through, one-half through, and entirely through stem) grouped <sup>2</sup> .....	{ do.....	1	4	40 stems.....	13	32.0	{ 10-60 20-90 90-100	{ 10-60
		2	4	40 stems.....	12	30.0		
		5	4	40 stems.....	18	45.0		
		10	4	40 stems.....	26	65.0		
		50-100	4	40 stems.....	39	97.5		

<sup>1</sup> Viable bacteria isolated from only 1 out of 20 tested for survival in inoculated trichome hairs.

<sup>2</sup> Estimated percentage of tiny wounds infected.

<sup>3</sup> The different depths of wounds were grouped together because in failing to control the depth of penetration of the inoculum into the wound by the technique used wound depth lacked significance.

took place increased pressure was put on the pipette contents so as to expel a quantity of the bacterial suspension into the plant cell. Almost immediately after the withdrawal of the pipette what appeared to be a protoplasmic plug filled the wound opening and effectively protected the cell from loss through evaporation. A droplet of india ink was placed on the stem surface adjacent to the inoculated trichome to facilitate its location afterward. Such inoculated cells appeared not to be injured and practically always remained alive, as judged by the fact that protoplasmic streaming continued after the operation. In a few instances particles resembling bacteria could be seen moving in the protoplasmic stream within trichome cells in much the same fashion as do india-ink particles when injected into similar cells.

Not a single instance of gall formation was found in these tests when final observations were made (fig. 2), and 3 weeks after inocula-

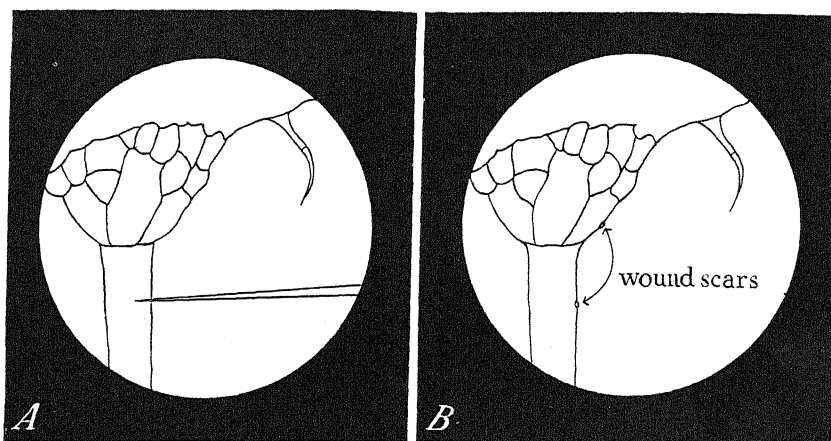


FIGURE 2.—Microinjection of individual trichome cells of tomato with crown gall bacteria: *A*, Many bacterial cells being injected into an individual cell (trichome or epidermal); *B*, at the end of 3 weeks no galls have resulted from the injection and no viable bacteria have been recovered.

tion, many of the trichome cells were still functioning normally. To account for these results several explanations are possible: (1) Wounds approximately  $3\mu$  in size may be too small to permit infection if it be assumed that a certain minimum quantity of wound hormone is essential for gall formation; (2) the intracellular environment may not be a favorable medium for these bacteria; (3) the injected bacteria that survived may have been too few or the period of survival too short for stimulating gall formation; or multiplication of bacterial cells, which apparently did not occur, may be essential for gall formation.

#### ISOLATIONS FROM INOCULATED TRICHOMES

Three weeks after inoculation, virulent crown gall bacteria were recovered from injected trichomes in but 1 case of 20 cultured. The isolations were attempted from 7, 6, and 7 living trichomes from 3 separate inoculation experiments and the crown gall organism was cultured from but 1 trichome in the first series. The hairs were removed by means of a tiny razor soldered to a needle and crushed in a droplet of broth on the inside of a test tube before washing into



a small quantity of sterile broth at the bottom. The interval of 3 weeks was thought to be sufficient for eliminating chance organisms left outside the wound opening in the inoculation operation. However, the single positive case may be the exception to the rule. Since bacteria were isolated in only 1 instance in 20 attempts it would seem that the intracellular environment was not a favorable medium for survival.

#### TINY GALLS PRODUCED AT TINY STEM WOUNDS

The smallest wounds in which infection occurred were produced by gently stroking the stems and petioles of tomato plants with a smooth polished needle previously moistened in a bacterial suspension of the crown-gall organism (fig. 3).

Within 5 days after inoculation tiny galls began to appear in close proximity to collapsed hairs and apparently arose from wounds involving one to several epidermal cells. The galls ranged from microscopic size to about 1 mm. in radial extension. Maximum size was reached within about 3 weeks, and forcing the plants by fertilization and planting in deep soil failed to materially increase the size of the galls even after 3 months.

It was estimated that less than 5 percent of the plant cells injured in this experiment became infected. The extent of the injuries depended on the pressure used in stroking and the number of times the needle was passed over a given point. The rubbing operation simultaneously applied a film of bacterial suspension and caused the extrusion of wound sap because of the pressure applied. As soon as

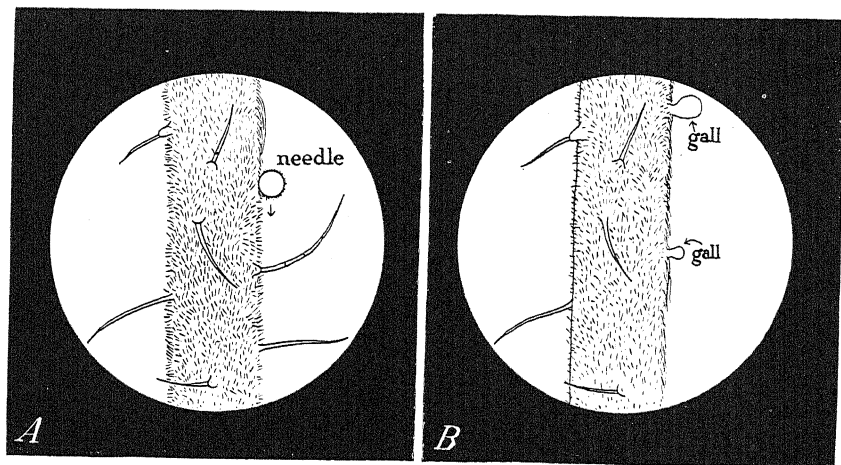


FIGURE 3.—Rubbing technique of inoculation: *A*, A needle dipped in bacterial suspension was stroked gently over the stem surface, resulting in minute wounds involving one to several plant cells; *B*, occasional tiny galls formed in proximity to the collapsed hair cells, such galls reaching a radial extension of about 1 mm.

the pressure was removed some of the sap and bacterial mixture returned into the wound.

From the results obtained it appeared that the small size of the galls may have been due to the minuteness of the food supply in the wounds and the relatively few bacterial cells that could gain entry and survive therein.

## BACTERIAL POPULATION IN TINY GALLS

Estimates of the number of bacterial cells were obtained by the standard dilution agar plate technique. Individual galls of several ages and sizes were used with or without a washing treatment to remove the bulk of chance organisms present on the outside. Excess water was blotted off the galls by means of sterilized paper toweling and entire galls were removed, cutting tangentially to the stem surface, with a razor. Each gall was ground fine in a sterile mortar in a small quantity of water before dilution plates were poured. It was soon found that dilutions were ordinarily unnecessary because of the small yield of bacteria. After an incubation period of about 4

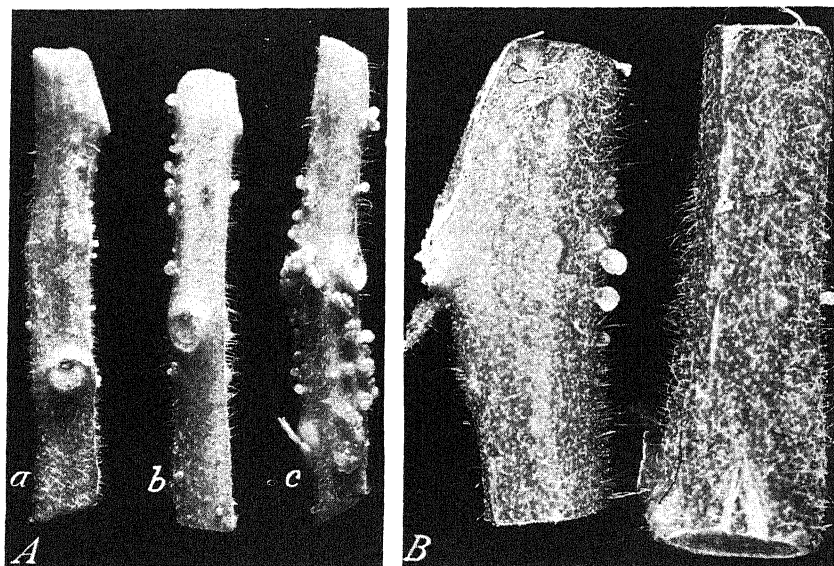


FIGURE 4.—A, Tiny galls induced by rubbing tomato stems with a polished needle gently (a) and progressively harder (b) and (c). The increase in size of wounds was correlated with an increase in the size of the galls induced.  $\times 0.9$ . B, Enlarged view of two stem pieces with similar tiny galls.  $\times 3$ .

days colonies resembling the crown gall organism were counted, and representative samples were used for pathogenicity tests to confirm the diagnosis.

On the whole, the number of bacteria isolated from tiny galls was very small, averaging less than 100. In 1 series of experiments the 54 galls cultured gave a total of 1,691 colonies, or an average of 31 colonies per gall, while the yield per gall ranged from 0 to 550 colonies. Only 4 galls yielded over 100 colonies. In another study 90 tiny galls yielded a total of 10,582 colonies or an average of 118 colonies per gall. Of these 1 gall yielded 5,520 or over half of the total; a second gall gave 1,223 colonies; 7 galls gave between 100 and 1,000 colonies; 34 galls less than 10 colonies; and 29 galls gave none. Failure to obtain any bacteria in about one-third of the galls suggests their elimination either through the technique employed or by death due to an unfavorable environment. It is possible that some of the galls negative for bacteria in the isolation tests are representative of what

is obtained when crown gall bacteria get inside living tomato cells or, as in the case of sunflower (18), when bacteria-free secondary galls occur at a distance from the point of inoculation. While the inability of crown gall bacteria to survive within injected cells, already mentioned, supports this conclusion, more work is needed on this point.

#### LOCATION OF BACTERIA IN THE GALLS

The approximate location of the crown gall bacteria in the tiny galls was studied by pouring plates of galls divided into two or three parts under the microscope by means of a microtool fashioned from a safety razor blade. Three experiments were conducted.

In the first experiment isolations were attempted from the tip one-third part of 7 tiny galls, and the results were positive in 2 cases. At the same time isolations made from 11 complete galls gave positive results in all cases.

The second experiment involved isolations from 10 tiny galls divided into 2 approximately equal parts. All galls yielded bacteria—the tip half in 9 cases and the basal half in 4 cases.

In the third experiment eight galls were divided into three approximately equal parts. Of the eight galls seven yielded pathogenic bacteria. Bacteria were obtained from the tip, middle, and basal regions respectively of three, five, and four of these tiny galls.

The results of the foregoing tests show that bacteria may be found in various parts of the tiny galls and are not limited to one location.

#### SMALLEST NUMBER OF BACTERIA REQUIRED TO INDUCE GALL FORMATION

##### SHALLOW NEEDLE-PUNCTURE WOUNDS

When one or more bacterial cells were used as inoculum and shallow stem wounds (about 0.1 mm. in diameter) of three depths (2 to 4 cells deep, 5 to 8 cells deep, and 10 to 12 cells deep, approximately) were employed as infection courts, infection occurred in a maximum of 21 percent of the trials (table 1).

Single bacteria induced gall formation in about 10 percent of the plants inoculated, as compared with approximately 15 percent for 2 to 10 bacterial cells and 21 percent for 50 to 100 bacterial cells (fig. 5).

The galls induced by single cell inoculation began to appear in about 1 week after inoculation and reached maximum size in 3 weeks. They were usually small, ranging from less than 1 to about 4 mm. in radial extension at the end of 3 months (fig. 6A). There was a tendency for the galls to become larger as the depth of the wound increased.

The galls induced by inoculation with 2 to 10 bacterial cells finally reached between 1 and 5 mm. in radial extension. Again the larger galls were associated with the deeper wounds.

When between 50 and 100 bacterial cells were introduced into shallow wounds the majority of the resulting galls reached between 1 and 7 mm. in radial extension and were larger for the greater wound depths.

From these results it is apparent that infection was somewhat higher and the galls were larger when more than one bacterial cell was used as inoculum and deeper wounds were inoculated. This slightly higher percentage of infection where more than one bacterial cell was used, while significant, is believed to be the result of chance. The probability of a single bacterium, as contrasted with two or more cells, reaching

the proper location in the wound for setting up an infection was undoubtedly less; therefore the percentage of infections would be expected to be smaller for the single cell. Moreover, one or more cells of an inoculum consisting of several cells would have a better chance of reaching a greater depth, as well as a better position for multiplication, than would a single cell. Consequently a correlation between size of gall and depth of wound was expected. Basic to the above interpretation is the well-known tendency of the crown gall

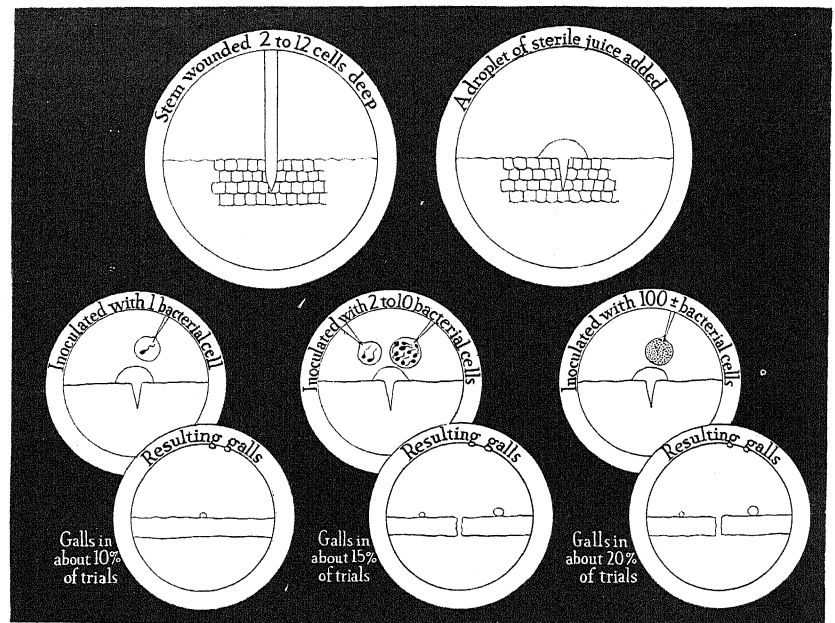


FIGURE 5.—Inoculation of shallow needle-puncture wounds with one or more crown gall bacteria, showing production of small galls.

organism to localize in the tissue and to stimulate only a limited number of the surrounding cells to activity.

#### DEEP NEEDLE-PUNCTURE WOUNDS

Deep wounds made by using the same size needle as before proved much more efficient as infection courts than shallow wounds (table 1). Three types of deep wounds (needle punctures one-fourth, one-half, and completely through the stems) were tested, one or more bacterial cells being used for inoculum. Single bacteria produced infection in from 10 to 60 percent of the trials (fig. 7); 2 to 10 bacteria produced galls in from 20 to 90 percent of the trials, and 50 to 100 bacteria produced galls in practically all the trials. In every case the galls were larger in radial extension than the thickness of the stems. There was a definite correlation between depth of wound and size of gall since the largest galls were always associated with needle punctures all the way through the stems (fig. 6, B). However, no apparent correlation was found between amount of inoculum and



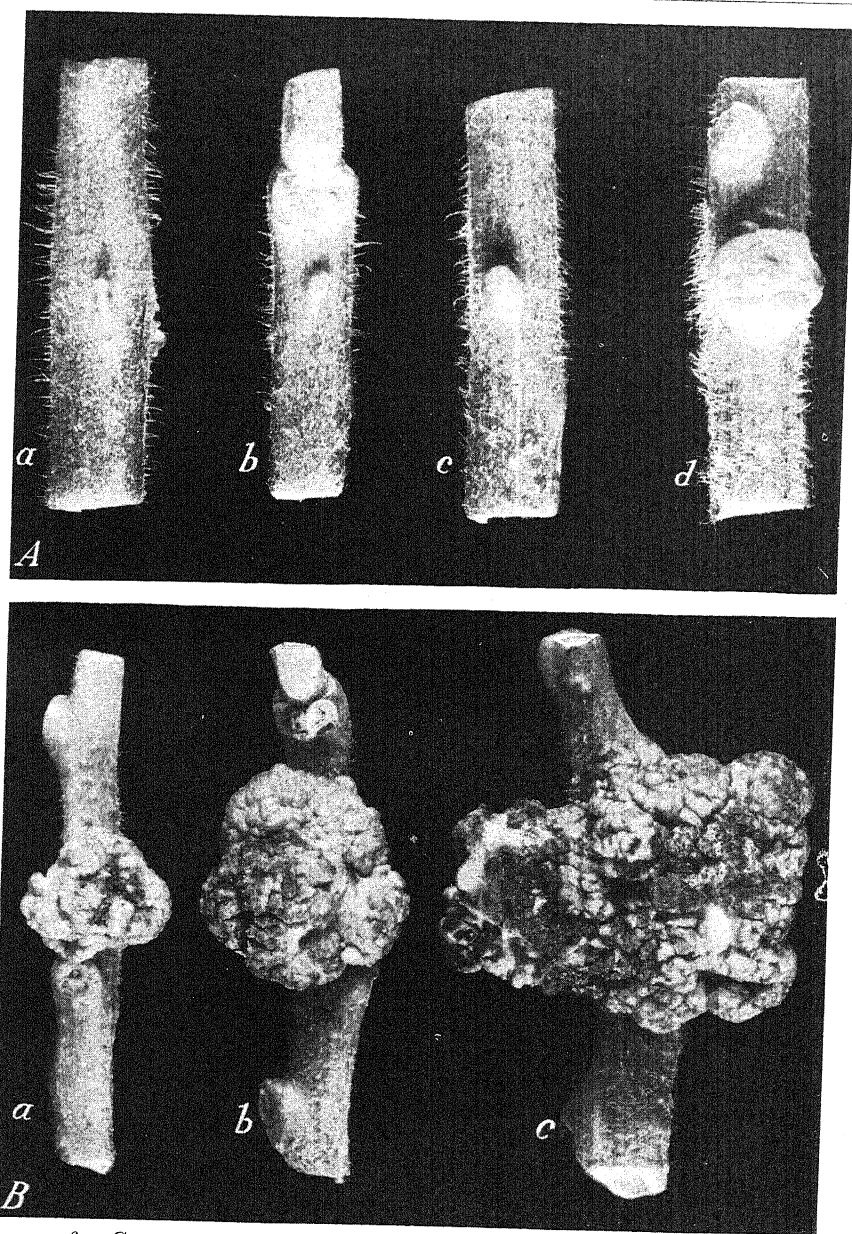


FIGURE 6.—Crown galls induced by inoculation with single bacteria: A, Maximum size attained in 3 months by crown galls induced by single-cell inoculation into wounds 2 to 4 cells deep (b); 5 to 8 cells deep (c); and 10 to 12 cells deep (d), as compared with a tiny gall (a) induced by inoculation of a very small epidermal wound resulting from the removal of a single trichome hair.  $\times 1.4$ . B, Maximum size attained at the end of 3 months by galls induced by the introduction of single bacteria into wounds one-fourth (a), one-half (b), and completely through (c) the tomato stem.  $\times 0.9$ .

size of gall. The reason for the single cell being less infective than larger numbers of cells on a percentage basis was undoubtedly due to the fact that there was less chance of one cell finding a favorable place for multiplication in the wound cavity. However, if a bacterium once found congenial surroundings, as in a deep wound, it proved actually equal in potentialities to larger numbers of cells so far as gall size, the end result, was concerned.

It has already been shown that one bacterium can quickly multiply into large numbers in sap extract and that the sap in the wound cavity functions in the same way as the food supply in microculture or in a test tube. The principal limiting factor governing gall size in these experiments was food supply for the increase of the bacterial population and not the initial amount of inoculum. Therefore, it was

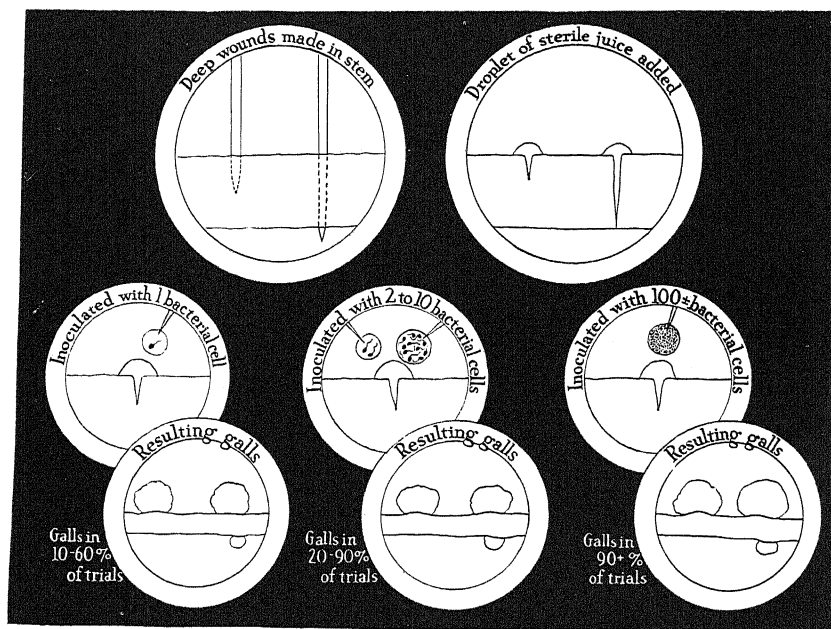


FIGURE 7.—Inoculation of deep needle-puncture wounds with one or more crown gall bacteria showing production of large galls.

concluded that the kind of wound was a more important factor in producing infection than the initial number of bacteria.

#### BACTERIAL POPULATION AND SIZE OF GALL

Isolations made from galls of various sizes taken at random from the several experiments in which shallow and deep needle-puncture wounds were employed indicated that bacterial population is roughly proportional to gall size. Small galls ranging from 1 to 3 mm. in radial extension yielded an average of between 50 and 100 colonies per gall in isolation trials. Always the entire gall was crushed in water in a Petri dish and allowed to stand for an hour before the agar was added. Galls from 5 to 7 mm. yielded between 100 and 50,000 colonies.

Galls 7 mm. and upwards ordinarily gave counts averaging 100,000 or more. One series of 7 galls measuring from 10 to 25 mm. in diameter yielded in round numbers 1,106,500; 400,000; 1,143,000; 1,070,000; 510,000; 11,000,000, and 20,000,000 bacteria based on the plate counts.

#### DISCUSSION

The present investigation, which involved the use of micrurgical technique, demonstrated the fact that a single cell of the crown gall organism when introduced into a wound is capable of producing infection in tomato. Apparently such potentiality of individual bacteria had been previously demonstrated in but one instance in animals (16) and one in plants (5).

The greatest difficulty encountered in this study was not that of developing precision technique for isolating and transferring known numbers of bacteria to the wounds, but rather that of introducing the isolated bacterial cells into the proper position in the needle-puncture wounds for infection to take place. Filling the wounds with sterile juice extract and then planting the inoculum on the protruding meniscus was found to be inadequate for the proper distribution of inocula in the shallow wounds, but proved much more efficient for the deep wounds. The interpretation given to this seeming discrepancy was that of the role of chance in distributing the bacteria in the wounds.

The complete failure of microscopic pipette wounds to become infection courts probably cannot be charged to lack of perfection of the microinjection technique. This conclusion is supported by the evidence from isolation experiments in which it was found that virulent cultures failed to survive the intracellular environment. Thus far no one has given convincing evidence that living crown gall bacteria are ever present in living plant cells. One of the most recent studies was that of Banfield (1) who reported negative evidence on this point. The final answer to this question will require further work.

#### SUMMARY

Juice extract from tomato plants was found to be an excellent medium for culturing the crown gall organism. Single bacteria grew readily in the juice extract in microculture.

The sap in the wound cavity liberated from the cells that were injured in the wounding operation also supported growth of the crown gall organism. Isolations made from wounds about 5 days after inoculation and before symptoms appeared, showed that the original single cells had multiplied into thousands of individuals in the wound sap.

The crown gall organism, when injected into the living cells of tomato stems, failed to induce gall formation and ordinarily failed to survive inside the cells, indicating that the living cell interior is an unfavorable medium for these bacteria.

Single bacterial cells induced gall formation when introduced into needle-puncture wounds of various sizes. The lower percentage of infections resulting from single-cell inoculation as contrasted with that from inocula consisting of larger numbers, was attributed to the role played by chance in distribution which favored the larger num-



bers reaching the proper position for multiplication and cell stimulation in the wounds.

Tiny wounds in the tomato stem, involving one or more epidermal cells, approximated the minimum size for infection by the crown gall organism. Only a small percentage of such wounds became infected when the inoculum was applied by gently rubbing the stem surface with a polished needle moistened with a bacterial suspension.

Shallow stem wounds (from about 2 to 12 cells deep) were less efficient as infection courts than deep wounds (from one-fourth to completely through the stem) when the inoculum was identical. This result was attributed to the role played by chance in the distribution of the bacteria and to the larger amount of wound sap which favored the larger wounds as infection courts.

Ordinarily the ultimate size of the gall was correlated with the depth of the wound but was independent of the size of the initial inoculum. The largest galls observed resulted from inoculating deep stem wounds regardless of whether the inoculum was a single bacterium or large numbers of bacteria.

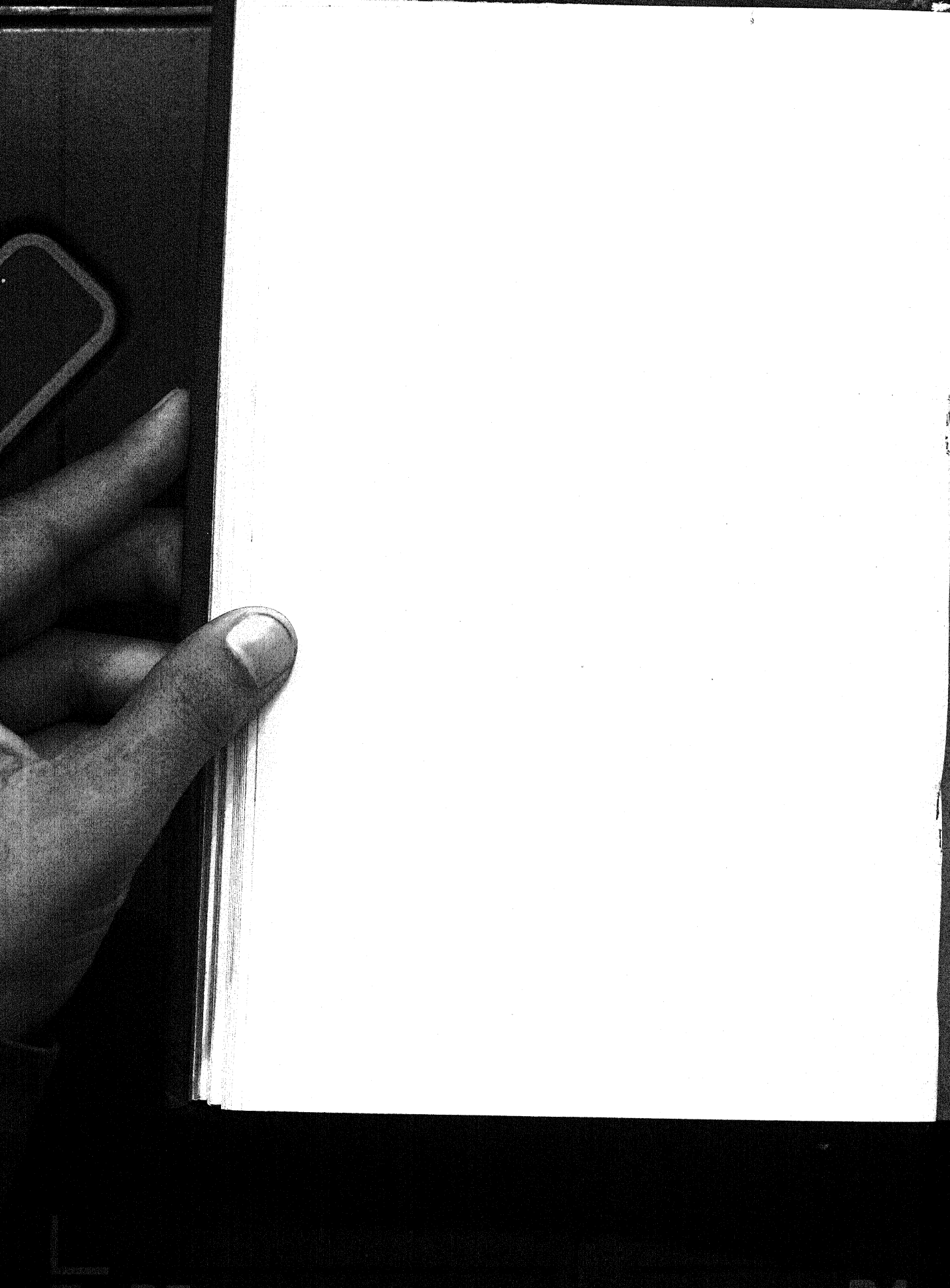
The relation (1) between tiny wounds, tiny galls, and few bacteria, (2) between shallow wounds, small galls and more bacteria, and (3) between deep wounds, large galls, and many bacteria was verified by isolation experiments.

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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Nitrogen Fixation by Azotobacter as Influenced by Molybdenum and Vanadium (Key No. G-1260) - - - - -	173
C. KENNETH HORNER, DEAN BURK, FRANKLIN E. ALLISON, and MILDRED S. SHERMAN	
Effects of Some Mild Forms of Mosaic on Potato and a Few Other Plants (Key No. Vt.-7) - - - - -	195
WINONA E. STONE	
Variability of Eroded Material (Key No. S-4) - - - - -	209
J. C. S. SLATER and E. A. CARLETON	



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NITROGEN FIXATION BY AZOTOBACTER AS INFLUENCED BY MOLYBDENUM AND VANADIUM<sup>1</sup>

By C. KENNETH HORNER, *junior chemist*, DEAN BURK, *formerly chemist*, FRANKLIN E. ALLISON, *senior chemist*, and MILDRED S. SHERMAN, *assistant chemist*, Division of Soil Microbiology, Bureau of Plant Industry, United States Department of Agriculture

## INTRODUCTION

The stimulating action of molybdenum on nitrogen fixation by *Azotobacter*, first reported by Bortels (7),<sup>2</sup> has since been observed by a number of other investigators. In addition, the beneficial effect of this element has been demonstrated by Bortels (12), for nitrogen fixation by *Nostoc*; by Steinberg (36, 37) and Steinberg and Bowling (39), for the growth of *Aspergillus niger*; by Bortels (10), Obratzova and others (32), Bobko and Savvina (6), and Bertrand (4), for legumes; and by De Rose, Eisenmenger, and Ritchie (19), Arnon (1), Arnon and Stout (2), and Piper (33), for other higher plants. Van Niel (40), Bortels (9), and Konishi and Tsuge (27, 28) have found that the addition of molybdenum or vanadium to soils may increase bacterial numbers and nitrogen fixation. Less is known about the effect of vanadium than of molybdenum on organisms; but whenever direct comparisons have been made on *Azotobacter* or legumes, vanadium has had a smaller effect than molybdenum on nitrogen fixation (8, 9, 10, 13, 15).

Previous investigations by Burk (13) and Burk and Horner (15, 16) of the influence of molybdenum and vanadium on *Azotobacter* consisted chiefly of growth-rate experiments with the Warburg manometric technique. Those studies showed that in a nitrogen-free medium growth and nitrogen fixation were increased markedly by molybdenum or vanadium but that these catalytic elements did not appreciably affect growth when the medium contained fixed nitrogen. A very low concentration of either molybdenum or vanadium was effective.

Other investigators who have reported studies of the effect of molybdenum or vanadium on *Azotobacter* have used the usual Erlenmeyer flask culture technique almost exclusively. The outstanding studies of this type are those of Bortels (8, 11), who determined the effect of varying concentrations and combinations of molybdenum, vanadium, iron, and other elements on nitrogen fixation or utilization and on pigment production, and those of Krzemieniewski and Kovats (30) and Kovats (29), who investigated the relative effects of iron, molybdenum, vanadium, humic acids, and the ash of the humates on nitrogen fixation and on efficiency of sugar utilization.

Experiments conducted with the Warburg respiration technique and with the stagnant long-time culture method are not necessarily directly comparable. The purpose of the studies reported herein was (1) to

<sup>1</sup> Received for publication October 13, 1941.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 191.

attempt to confirm some of the results obtained by others, particularly those of the investigators mentioned in the preceding paragraph, by using their methods, and (2) to obtain information on other phases of the problem not yet studied or inadequately studied. These latter include the influence of molybdenum in relation to age of culture, its impurity in the media, and strain variation. The effective concentration range of both molybdenum and vanadium for several typical strains is given. These data are needed for a proper evaluation of previous work and for a better understanding of the nutritional or catalytic role of these elements. The question whether molybdenum and vanadium are specific catalysts for the nitrogen-fixation process or whether they also favor the growth of *Azotobacter* supplied with combined nitrogen will not be considered here. Aside from workers in this laboratory (13, 14, 15, 16), only Birch-Hirschfeld (5) and Bortels (8) have considered this phase of the problem. This subject is now under active investigation. In all the work reported below, the organisms were grown on nitrogen-free media, where growth is dependent upon nitrogen fixation; hence the two terms "growth" and "nitrogen fixation" are used interchangeably.

#### MATERIALS AND METHODS

The two culture media chiefly employed are designated as A and B. Medium A was prepared as follows: Eight-tenths of a gram of dipotassium phosphate ( $K_2HPO_4$ ), 0.2 gm. of monopotassium phosphate ( $KH_2PO_4$ ), 0.2 gm. of magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ), and 0.1 gm. of calcium sulfate ( $CaSO_4 \cdot 2H_2O$ ) were added to 1 liter of distilled water and thoroughly shaken. After the undissolved material had settled, the clear solution was decanted off as desired. It contained about 15 percent less phosphate and 40 percent less calcium than had been added. Just before use, an additional 20 percent of distilled water, 10 or 20 p. p. m. of synthetic humate (containing 10 percent of iron) (25), 0.05 percent of neutralized lactic acid,<sup>3</sup> and 1 or 2 percent of sucrose or glucose were added to the clear solution. The constituents of this medium remain soluble so that daily observations of bacterial growth or turbidimetric analyses can be made. Medium B was similar to that of Bortels and was prepared by adding 1.0 gm. of dipotassium phosphate ( $K_2HPO_4$ ), 0.5 gm. of magnesium sulfate ( $MgSO_4 \cdot 7H_2O$ ), 1.0 gm. of calcium carbonate ( $CaCO_3$ ), 0.02 gm. of iron sulfate ( $FeSO_4 \cdot 7H_2O$ ), and 10 or 20 gm. of glucose or sucrose to 1 liter of distilled water. Medium B was always turbid because of the excess of calcium carbonate. When molybdenum was added to either medium it was supplied as sodium molybdate ( $Na_2MoO_4 \cdot 2H_2O$ ); vanadium was added as the ortho or meta vanadate. Modifications of media A and B that were used in some experiments are described in connection with the individual experiments.

Erlenmeyer flasks of 125- or 250-cc. capacity, containing 15 or 25 cc. of media, respectively, served as culture vessels. This gave a layer of liquid 3 to 6 mm. in depth. The flasks were plugged with cotton and sterilized in an autoclave at 10 to 15 pounds' pressure for 15 to 30 minutes. When glucose was present, the lower pressure and shorter time were used to prevent excessive decomposition.

<sup>3</sup> A small amount of sodium lactate has been found to be advantageous in maintaining a slightly alkaline reaction for some strains of *Azotobacter* that tend to produce acidity. The pH of the cultures varied between 6.6 and 8.6; in general, cultures with the most growth had the highest pH values.

The two strains of *Azotobacter* employed in the majority of the experiments were *A. chroococcum* Beij. B-8<sup>4</sup> and *A. vinelandii* Lip. V-1.<sup>5</sup> Other strains, used for comparison, were *A. chroococcum* B-5, B-7, and B-10;<sup>4</sup> *A. vinelandii* B-4, B-6, and B-9;<sup>4</sup> *A. agile* Beij. K-1 (26);<sup>6</sup> *A. agilis atypica* Kluyver and Van den Bout K-2;<sup>6</sup> *A. chroococcum* C-4 and C-5;<sup>7</sup> and *A. chroococcum* C-12 and C-15.<sup>8</sup>

Inoculation of the media in the experimental flasks was accomplished by adding one or two drops of a 1- or 2-day liquid culture that had been growing for some time on medium A with frequent transfers. The cultures were incubated at 28° to 31° C. for various periods, depending upon the purpose of the experiments. The purity of the cultures, especially of the inoculum, was checked by frequent inoculation into a peptone-meat extract medium as well as by microscopic examination.

Analyses were made on aliquots of the cultures at the end of the incubation period after sufficient distilled water had been added to restore them to their original volume. The dry matter produced was obtained by centrifuging, washing once with distilled water, and drying in an electric oven at 80°-100° C. The unoxidized carbohydrate in the centrifugate was determined by means of the Von Fellenberg (20) chromic acid oxidation procedure. This method, though not strictly specific for the determination of sugar, is convenient and gives a close approximation in the case of *Azotobacter*, which is known to oxidize glucose and sucrose or their intermediate oxidation products almost completely to CO<sub>2</sub> and H<sub>2</sub>O (31). Centrifugates from cultures that have obtained maximum growth usually give a reaction, upon chromic acid oxidation, equivalent to up to 5 percent of the initially added sugar. This is probably non-sugar and largely represents carbon originating from the excreted nitrogenous compounds (24). The oxidizable carbon was calculated as glucose on the basis of 1 cc. of 0.1 N potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) as equivalent to 0.75 mg. of glucose. All nitrogen, dry-matter, and carbohydrate analyses were based on 100 cc. in order to facilitate comparisons. Turbidimetric measurements were made by means of a Bausch and Lomb nephelometer. The standard for comparison was prepared by shaking an excess of pulverized bentonite in distilled water, allowing it to stand overnight, and using the decanted suspension or suitable dilutions of it. Humate was added, comparable to that in the culture medium, to provide approximately the same tint. The most turbid standard was arbitrarily called 800 units, which is equivalent to the turbidity given by a culture of *Azotobacter* containing about 14 to 18 mg. of nitrogen or 100 to 150 mg. of dried bacteria per 100 cc. These turbidity units are not necessarily comparable with those reported in previous publications from this laboratory. Colorimetric pH determinations were made with various La Motte indicators and standards. Total nitrogen was determined by the micro-Kjeldahl method.

<sup>4</sup> Obtained from O. P. Maximova, of the laboratory of A. N. Bach, U. S. S. R. Academy of Sciences, Moscow.

<sup>5</sup> Original strain isolated by J. G. Lipman and obtained from him about 18 years ago.

<sup>6</sup> Received from H. W. Reuszer, formerly of the Colorado Agricultural Experiment Station, who had obtained them from A. J. Kluyver, of Delft, Netherlands.

<sup>7</sup> Obtained from the National Collection of Type Cultures, Lister Institute, London, England, as Nos. 4183 and 1865.

<sup>8</sup> Obtained from N. R. Smith, of the Division of Soil Microbiology, Bureau of Plant Industry, U. S. Department of Agriculture, as Nos. 12 and 15.

## EXPERIMENTAL RESULTS

## AGE OF CULTURE

Figures 1 and 2 show the effect of molybdenum upon nitrogen fixation by *Azotobacter chroococcum* B-8 and *A. vinelandii* V-1 in relation to age of culture. The data presented in these figures are from numerous experiments carried out over a period of 3 years.

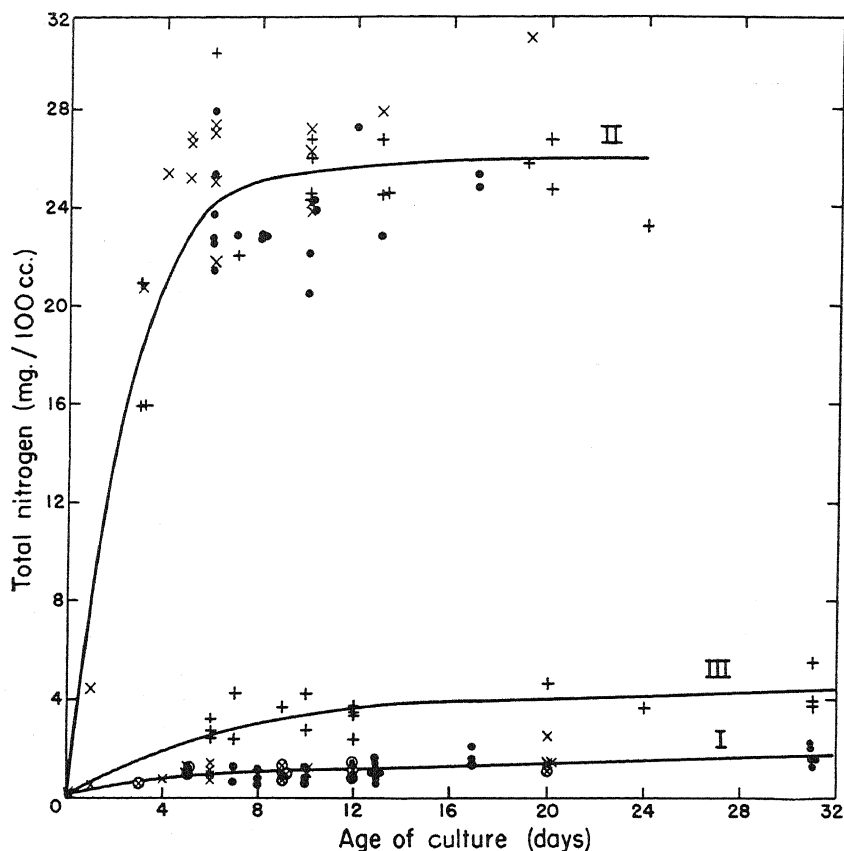


FIGURE 1.—Total nitrogen fixed by *Azotobacter chroococcum* B-8 as a function of age, with and without added molybdenum: Curve I, no added molybdenum, and curve II, 1 or 4 p. p. m. of molybdenum, various samples of sucrose or glucose serving as sources of energy; curve III, no added molybdenum, sucrose 1 serving as the source of energy. Symbols: • = 2 percent glucose; ○ = 1 percent glucose; + = 2 percent sucrose 1; × = 2 percent other sucroses; ⊗ = 1 percent other sucroses.

Although the conditions in the various experiments were similar, both media A and B with modifications were employed, and several samples of chemically pure sucrose or glucose designated in the legends were used. The usual experimental variations further tended to give a scattering of points around the curves.

The preponderant influence of molybdenum over any of these other variables is evident from the extreme divergence (a maximum of



thirtyfold to fortyfold) between curves I and II. Curve III of figure 1 indicates that one sample of sugar, sucrose 1, which consistently caused 50 to 100 percent or more growth than any of the other energy sources in the absence of added molybdenum, probably contained an appreciably greater impurity. Further evidence for this will be given, below. Both strains of *Azotobacter* responded similarly to molybdenum

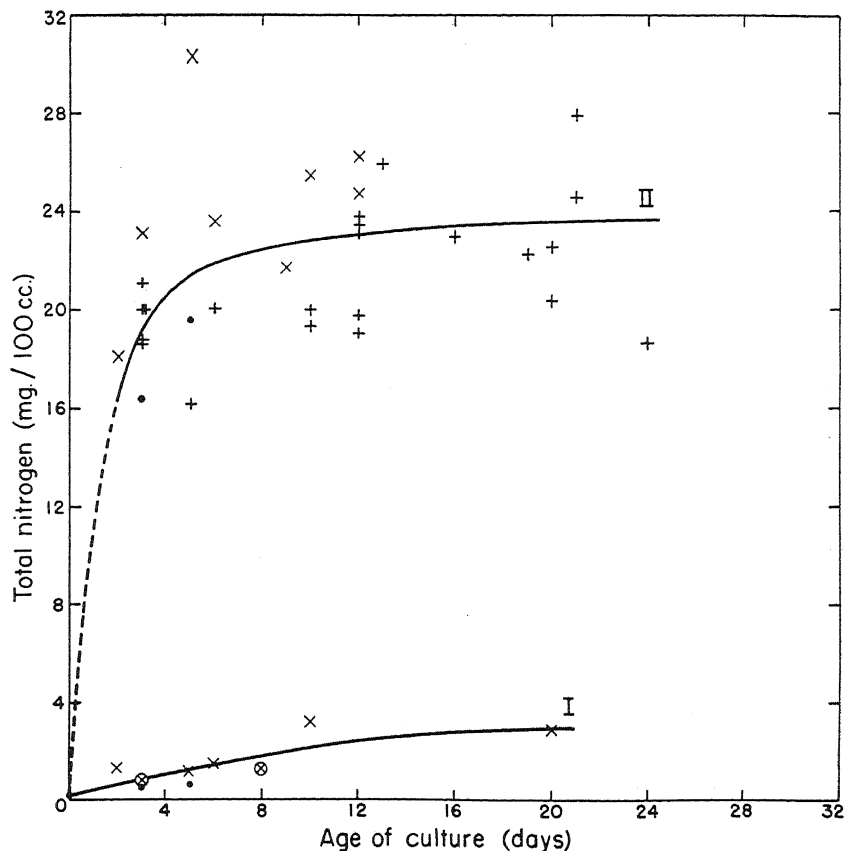


FIGURE 2.—Total nitrogen fixed by *Azotobacter vinelandii* V-1 as a function of age, with and without added molybdenum: Curve I, no added molybdenum, and curve II, 1 or 4 p. p. m. of molybdenum, various samples of sucrose or glucose serving as the sources of energy. Symbols: • = 2 percent glucose; + = 2 percent sucrose 1; × = 2 percent other sucroses; ⊗ = 1 percent other sucroses.

attaining maximum growth by about the sixth to eighth day. In the absence of added molybdenum, analyses have shown 1 to 1.5 gm. out of the original 2 gm. per 100 cc. of carbohydrate still remaining after 31 days. Since the energy supply is not limiting in the latter case, cultures with either 1 or 2 percent fall equally well on curve I. The cultures without molybdenum continue to grow slowly for weeks after those with adequate molybdenum have consumed their energy supply and ceased growing, but the greatest increment in nitrogen

fixation takes place during the first few days. This would seem to indicate that if no molybdenum impurity were present there would be no growth. *A. vinelandii* V-1, as a rule, gives a little more nitrogen fixation with no added molybdenum and a slightly lower maximum when it is optimum than is observed for *A. chroococcum* B-8; the latter organism also usually grows somewhat more rapidly during the first 1 or 2 days, at least as evidenced by turbidity observable.

The relation of age of culture to total nitrogen fixed by another strain, *Azotobacter vinelandii* B-6, in the presence and absence of

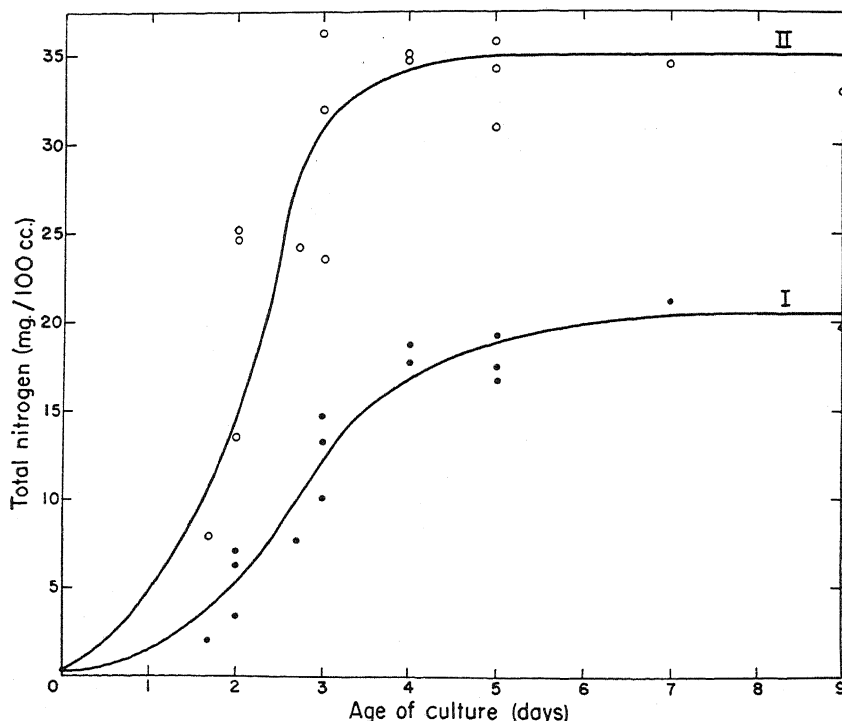


FIGURE 3.—Total nitrogen fixed by *Azotobacter Vinelandii* B-6 as a function of age, with and without added molybdenum; energy source, 2 percent sucrose 2: Curve I, no added molybdenum; curve II, 1 p. p. m. molybdenum.

molybdenum, is shown in figure 3. The maximum nitrogen fixation with 2 percent of sugar and adequate molybdenum was only a little greater for this strain than for *A. vinelandii* V-1 (fig. 2), but in the absence of molybdenum the relatively large amount of growth of the former in contrast to the latter is very striking. In figure 3 it will be observed that on the second or third day the fixation in the absence of added molybdenum was 30 to 40 percent of that with molybdenum; on the fifth to sixth day it was 50 percent or more. Such high nitrogen fixation in the presence of mere traces of molybdenum as impurity, amounting to 20 to 22 mg. of nitrogen per 100 cc., in contrast to 1 to 2 mg. of nitrogen fixed by the other two organisms, shows a very different molybdenum requirement for this organism.

## MODIFICATION AND PURIFICATION OF MEDIA

Several attempts were made to lower the molybdenum impurity in the media and to obtain more convincing proof of its essentiality for nitrogen fixation by *Azotobacter*. In the writers' earlier work, 0.2 mg. of sodium chloride (NaCl) per liter had been added to medium A, but recent tests have shown not only that it is unnecessary but that it may contain a trace of molybdenum. It was also observed that freshly prepared medium A usually gave somewhat less growth in the absence of molybdenum than if this medium were prepared from inorganic solutions that had stood for some time in contact with the undissolved sediment. The use of water that had been redistilled

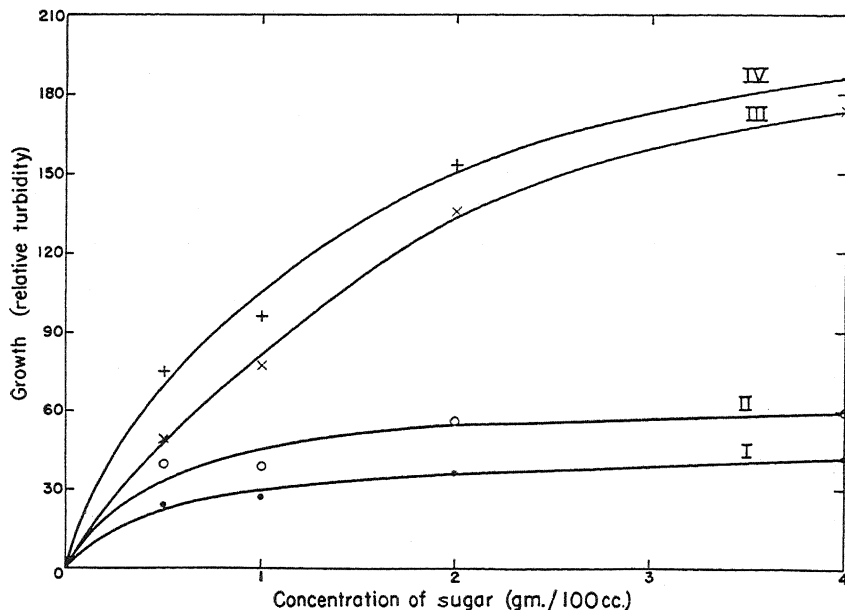


FIGURE 4.—Growth of *Azotobacter chroococcum* B-8 as a function of sugar concentration in the absence of added molybdenum: Curve I, sucrose 2, 2-day culture; curve II, sucrose 2, 5-day culture; curve III, sucrose 1, 2-day culture; curve IV, sucrose 1, 5-day culture.

from pyrex vessels gave the same results as ordinary distilled water. The sources of calcium or iron were reversed for the two media in some experiments, and other sources were tested, but there was no consistent effect either on the maximum nitrogen fixation by *Azotobacter* obtainable with optimum molybdenum or on the minimum without molybdenum.

The sugar, which is present in many times the concentration of any other constituent of the media, may well furnish considerable molybdenum impurity, as is indicated in figure 1. Out of about a dozen different c. p. sugars tested, including sucrose, glucose, mannite, and levulose, all except sucrose 1 gave about the same small growth on molybdenum-deficient media. Unfortunately, this particular sugar had been in use for some time before it was compared with other samples. Figure 4 shows the striking difference in the response of

*Azotobacter chroococcum* B-8 to increasing concentrations of sucrose 1 and sucrose 2. The latter caused less than a twofold increase in growth as the concentration was raised from 0.5 to 4 percent, whereas the former gave a threefold effect. The relatively small change between the 2- and 5-day cultures indicates little tendency for the cultures with sucrose 2 to approach with age those with sucrose 1, at least not while the energy supply is adequate. In a similar series of cultures, where optimum molybdenum was added and heavy growth and a rapid utilization of sugar occurred, there was no essential difference between the growths with the two sugars. It would seem, therefore, that the improved development of molybdenum-deficient cultures produced by sucrose 1 is due to a greater molybdenum impurity. A concentration of sugar greater than 4 percent tends to inhibit growth of *A. chroococcum* B-8, with or without molybdenum, and hence the shape of the curves in figure 4 may be influenced to some extent by an approach to this inhibiting range.

TABLE 1.—Nitrogen fixation by 10-day cultures of *Azotobacter chroococcum* B-8 as affected by charcoal purification of sugar and salts of the medium

Sucrose No.	Sugar (2 percent) purified	Inorganic salts purified	Total nitrogen (mg. per 100 cc.) with—		Sucrose No.	Sugar (2 percent) purified	Inorganic salts purified	Total nitrogen (mg. per 100 cc.) with—	
			No Mo	1 p. p. m. Mo				No Mo	1 p. p. m. Mo
3	—	—	1.29	26.0	3	+	+	1.02	—
3	+	—	.99	26.2	3	+	+	1.00	23.8
3	—	+ <sup>1 2</sup>	1.32	—	3	+	+	.93	—
3	—	+	1.36	—	1	—	+	4.23	24.5
3	—	+ <sup>1</sup>	1.34	24.0	1	+	+	1.03	24.1
3	+	+ <sup>1 2</sup>	.43	—	1	+	+	1.17	—

<sup>1</sup> Calcium was supplied as  $\text{CaCl}_2$  instead of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .

<sup>2</sup> Salt solutions collected after filtering through the charcoal 2 to 3 hours; others collected during first 2 to 3 hours.

<sup>3</sup> Not treated with charcoal but recrystallized from water with alcohol.

<sup>4</sup> Purified with Planstiehl charcoal; others purified with Norit.

Preliminary attempts to remove this apparent molybdenum impurity from the media by means of Steinberg's (35) procedure of calcium carbonate ( $\text{CaCO}_3$ ) precipitation or by letting *Azotobacter* remove the impurity and then re-using the media were unsuccessful. However, if sugar solutions were allowed to percolate through charcoal, and the sugar was then recrystallized from alcohol, the growth of *Azotobacter* in the media containing the purified sugars and no added molybdenum was definitely lowered. When optimum molybdenum was added to the media the charcoal treatment of the sugars caused no significant effect on the growth of the organisms. Table 1 summarizes the results of an experiment with *Azotobacter chroococcum* B-8 in which various combinations of purified and unpurified salts and sugars were employed as media. The charcoal treatment of the salts had practically no effect, but the purified sucrose 1 gave markedly less growth in the absence of molybdenum than did the unpurified sample. Even with sucrose 3, slightly less growth in molybdenum-deficient media was obtained when this sugar was treated with charcoal, although recrystallization from alcohol alone was nearly as efficient. It is interesting that the nitrogen fixed by the organisms in the absence of molybdenum was nearly the same in the presence



of either of the purified sucroses 1 or 3, and only slightly less than with unpurified sucrose 2. Evidently it is the molybdenum impurity in the various samples of sucrose that is chiefly responsible for their different behavior in supporting nitrogen fixation by *Azotobacter*.

#### STRAIN VARIATION

The results of two experiments in which 14 strains, comprising 3 species of *Azotobacter*, were compared are shown in table 2. Data are given for total nitrogen fixed, dry matter, cell nitrogen, carbohydrate consumed, and milligrams of nitrogen fixed per gram of sugar utilized for cultures with and without added molybdenum. The two experiments are not strictly comparable because of the different incubation periods, concentrations, and samples of sucrose used. The lower concentration of sugar used in experiment 1 reduced the total molybdenum added as impurity but of course also limited the total nitrogen fixation in the presence of an adequate supply of this element. Bearing in mind these considerations, certain generalizations may be made. It will be noted that when no molybdenum was added the two strains of *Azotobacter agile* and all the strains of *A. vinelandii* except *A. vinelandii* V-1 gave relatively high nitrogen fixation. It would appear that even with sucrose 2, although only one of these strains in each of the two species was tested with the purer sugar, the total nitrogen fixation amounted to 60 to 70 percent of that obtained with optimum molybdenum. On the other hand, the strains of *A. chroococcum*, with sucrose 2, showed a nitrogen fixation in the absence of added molybdenum of about 10 percent or less of that in the presence of an adequate supply of this element. Thus, in this respect *A. vinelandii* B-6 appears to be a typical representative of the *A. agile* and *A. vinelandii* strains studied, and *A. chroococcum* B-8 behaves like the other strains of *A. chroococcum*.

Over a period of years *Azotobacter vinelandii* V-1, as mentioned in the discussions of figures 1 and 2, has usually responded to molybdenum more nearly like the *A. chroococcum* strains than like the other *A. vinelandii* organisms tested. In several recent experiments, however, cultures of this particular strain fixed 10 to 14 mg. of nitrogen per 100 cc. in the absence of added molybdenum. Normal behavior of cultures of *A. chroococcum* B-8, which were tested simultaneously, showed that the changed behavior of *A. vinelandii* V-1 was not due to a higher molybdenum impurity in the medium. Further work will be necessary to determine whether or not *A. vinelandii* V-1 has undergone a permanent variation with respect to the quantitative effect of molybdenum.

The strains of *Azotobacter chroococcum* whose behavior in respect to molybdenum was similar showed certain differences among themselves in other physiological characteristics. For example, strains B-10, C-4, C-12, and C-15 grew especially slowly under the conditions employed, even when supplied with molybdenum. This is evident from table 2, experiment 1, which shows that by the third day all the cultures except these had attained practically maximum growth. *A. chroococcum* C-15 appears to be unique in possessing a lower ultimate nitrogen-fixing ability. Whereas all of the other *A. chroococcum* strains in experiment 1 gave a fixation of 13 to 16 mg. of nitrogen per 100 cc. of medium by the eighth day, when growth had practically stopped, this organism yielded only 9.3 mg. of nitrogen per

100 cc., with carbohydrate still remaining and growth essentially at maximum. Table 2 also shows that the efficiency of carbohydrate utilization was normal. Perhaps this organism requires additional trace elements as reported for several strains of *Azotobacter* by Schröder (34).

TABLE 2.—Comparative effect of molybdenum (1 p. p. m.) on different strains of *Azotobacter*

[Figures are for 100 cc. of culture]

Experiment No. and <i>Azotobacter</i> species and strain	Age of culture	Total nitrogen fixed with—		Dry weight of cells with—		Nitrogen content of cells with—		Sugar consumed with—		Nitrogen fixed per gram of sugar consumed with—	
		No Mo		No Mo		No Mo		No Mo		No Mo	
		Mg.	Mg.	Mg.	Mg.	Pct.	Pct.	Gm.	Gm.	Mg.	Mg.
Experiment 1: <sup>1</sup>	Days										
<i>A. agile</i> K-1	3	7.58	10.7	86	101	7	9	1.07	1.06	7	10
<i>A. vinelandii</i> B-6	3	8.15	13.8	54	85	10	12	1.06	1.06	8	13
<i>A. vinelandii</i> V-1	3	.77	8.2	<sup>2</sup> 7	58	<sup>3</sup> 9	12	1.07	1.07	—	8
<i>A. chroococcum</i> B-5	3	.54	13.1	<sup>2</sup> 3	93	<sup>3</sup> 14	10	—	—	—	16
<i>A. chroococcum</i> B-7	3	.60	14.6	<sup>2</sup> 4	84	<sup>3</sup> 12	12	—	1.02	—	14
<i>A. chroococcum</i> B-8	3	.49	15.0	<sup>2</sup> 4	90	<sup>3</sup> 10	13	—	1.04	—	14
<i>A. chroococcum</i> B-10	3	.49	10.0	<sup>2</sup> 9	159	<sup>3</sup> 4	4	—	.65	—	15
<i>A. chroococcum</i> C-4	3	.37	5.4	<sup>2</sup> 4	43	<sup>3</sup> 8	10	—	.32	—	17
<i>A. chroococcum</i> C-5	3	.46	12.7	<sup>2</sup> 4	122	<sup>3</sup> 9	8	—	.86	—	15
<i>A. chroococcum</i> C-15	3	.49	6.2	<sup>2</sup> 7	49	<sup>3</sup> 6	11	—	.39	—	16
<i>A. agile</i> K-1	8	7.08	10.9	—	—	—	—	—	—	—	—
<i>A. vinelandii</i> V-1	8	1.26	10.0	11	59	8	12	.45	1.07	3	9
<i>A. chroococcum</i> B-5	8	.94	13.7	17	75	5	12	.14	1.04	7	13
<i>A. chroococcum</i> B-7	8	.77	—	<sup>2</sup> 13	—	<sup>3</sup> 5	—	.22	—	4	—
<i>A. chroococcum</i> B-8	8	1.00	14.7	14	86	6	12	.19	1.06	5	14
<i>A. chroococcum</i> B-10	8	.97	14.3	<sup>2</sup> 19	—	<sup>3</sup> 4	—	.38	—	3	—
<i>A. chroococcum</i> C-4	8	.77	15.5	<sup>2</sup> 13	108	<sup>3</sup> 5	12	.11	1.05	7	15
<i>A. chroococcum</i> C-5	8	.77	14.7	<sup>2</sup> 14	—	<sup>3</sup> 4	—	.14	—	6	—
<i>A. chroococcum</i> C-12	8	.90	13.0	<sup>2</sup> 7	—	<sup>3</sup> 9	—	—	—	—	—
<i>A. chroococcum</i> C-15	8	.80	9.3	21	68	3	10	.13	.66	6	14
Experiment 2: <sup>4</sup>											
<i>A. agile</i> K-1	6	10.0	26.0	110	174	9	13	1.00	1.82	10	14
<i>A. agilis atypica</i> K-2	6	7.2	14.3	—	—	—	—	—	—	—	—
<i>A. vinelandii</i> B-4	6	9.3	25.0	83	184	10	11	.80	1.70	12	15
<i>A. vinelandii</i> B-6	6	12.3	28.9	—	—	—	—	—	—	—	—
<i>A. vinelandii</i> B-9	6	9.3	19.6	—	—	—	—	—	—	—	—
<i>A. vinelandii</i> V-1	6	4.1	20.0	—	—	—	—	—	—	—	—
<i>A. chroococcum</i> B-8	6	2.7	16.5	—	—	—	—	—	—	—	—
<i>A. chroococcum</i> C-4	6	1.9	19.6	22	143	8	14	.26	1.52	7	13
<i>A. chroococcum</i> C-15	6	2.2	9.6	42	87	—	10	.17	.67	13	14

<sup>1</sup> Initial sugar, 1.10 gm. of sucrose 2 per 100 cc.

<sup>2</sup> Dry matter estimated from turbidity.

<sup>3</sup> Nitrogen content calculated on the basis of cell nitrogen equal to 80 percent of the total nitrogen.

<sup>4</sup> Initial sugar, 2.00 gm. of sucrose 1 per 100 cc.

The dry matter produced by the various strains was usually roughly parallel to the nitrogen fixed, except for *Azotobacter chroococcum* B-10 and C-5. The proportionately greater dry weight of these organisms was due to larger gum formation. To permit satisfactory centrifugation the cultures were acidified to pH 2 to 3, which coagulated the gum with the cells and accounts for the lower nitrogen content of the dry matter. Where molybdenum was present most of the cultures gave a rather uniform nitrogen content of 10 to 12 percent of the dry matter. Many of the molybdenum-deficient cultures were apparently considerably lower in their percentage of nitrogen, but the approximate estimation of both the dry matter and cell nitrogen for the very light cultures reduces the accuracy of the figures.

The efficiency of carbohydrate utilization (milligrams of nitrogen fixed per gram of carbohydrate consumed) varied to some extent for the different cultures but was frequently twofold to threefold greater with molybdenum. Bortels (8), Krzemieniewski and Kovats (30), and Kovats (29) report similar or somewhat greater differences for their organisms with and without molybdenum. This element, therefore, exerts its effect upon both the rate of growth of *Azotobacter* and the total nitrogen fixation, or growth per unit of sugar consumed, the latter very probably being largely a consequence of the former.

#### EFFECTIVE CONCENTRATION RANGE OF MOLYBDENUM AND VANADIUM

Figure 5 represents graphically the nitrogen fixation of 6-day-old cultures of *Azotobacter chroococcum* B-8 as a function of suboptimal concentrations of molybdenum and vanadium when sucrose 1 and sucrose 2 served as sources of energy. A comparison of the molybdenum curves shows that with the lower range of molybdenum there is considerable divergence between the results with the two sugars, but as the optimum is approached the curves tend to coincide. This is true also for the vanadium curves. If we assume that the nitrogen fixed with no added molybdenum depends upon the impurity in the medium, the apparent trend of all of the curves is to approach zero nitrogen fixation with zero molybdenum or vanadium, and we may extrapolate the curves to their intercept with the horizontal axis and obtain an approximate analysis of this impurity. The difference between the intercepts for the two curves ( $b-a$ ) would indicate the extra impurity supplied by sucrose 1. The estimates thus obtained indicate a maximum of about 0.0002 p. p. m. of molybdenum (or vanadium) contained in the medium with 2 percent of sucrose 2, and 0.0012 p. p. m. with 2 percent of sucrose 1. It is obvious that these are only rough estimates because of the nonlinearity of the curves and culture variabilities, but if the points for curve II are shifted along their horizontal axes for a distance equal to  $b-a$ , practically all fall on curve I. The same correction might be made with respect to vanadium for curves III and IV, but they do not so nearly coincide. This does not mean, however, that the impurity is solely molybdenum, for curves III and IV may be merely less accurately defined than curves I and II. The estimated 0.001 mg. of molybdenum supplied to the medium by 20 gm. of sucrose 1, while sufficient to yield about 10 percent of the optimum effect, is in reality only a minute trace (0.000005 percent) of impurity. It may be of interest to recall that Steinberg (38) reported the presence of 0.00004 percent of molybdenum in the sucrose he used.

The effective suboptimal concentration range for 6-day-old cultures of *Azotobacter chroococcum* B-8 covers the range from 0.0001 p. p. m. (detectable effect) to 1 p. p. m. (essentially optimum). A concentration of about 0.03 p. p. m. yields half-maximum growth, whereas 0.1 p. p. m. gives 80 to 90 percent of the maximum effect (fig. 5, insert). The concentration ranges for molybdenum and vanadium are practically identical, but, as reported previously (13), the latter element gives only about two-thirds of the maximum growth produced by molybdenum. The differences between the effects of the two elements at the lower concentrations tend to become somewhat smaller, especially when culturing with less pure sucrose. Bortels (8) has

reported very similar concentration ranges for his strains of *A. chroococcum* and *A. vinelandii*, especially at the upper ranges that have been studied most. His optimum concentration of vanadium appears to be somewhat less than that of molybdenum, and he observed toxicity with about 1 p. p. m. of vanadium. In the writers' experiments, no toxicity was observed at several times this concentration. In earlier work in this laboratory, in which the Warburg manometric technique was used for growth-rate studies of *A. vinelandii* V-1, a much lower and narrower effective range of molybdenum was obtained (0.000003 to 0.0001 p. p. m.). More recent experiments

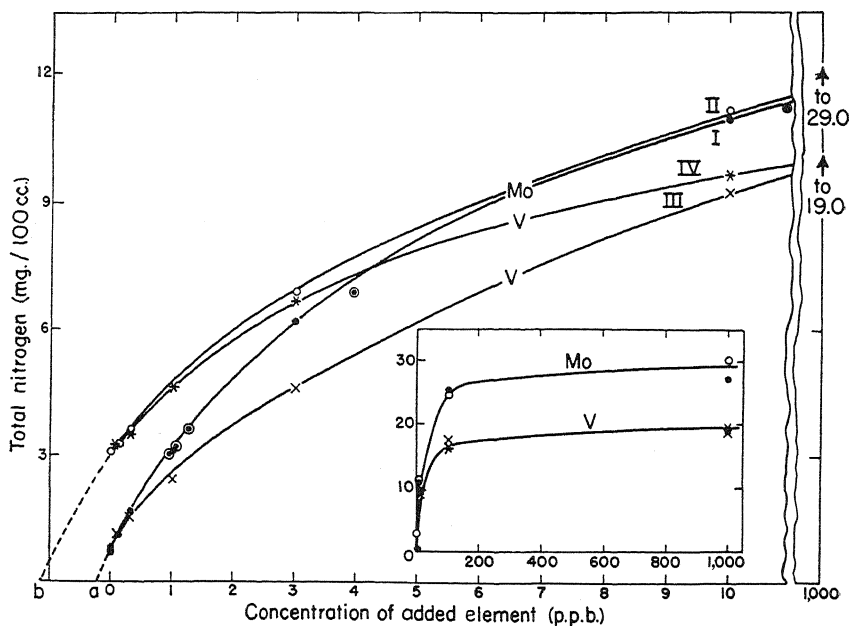


FIGURE 5.—Total nitrogen fixed by 6-day-old cultures of *Azotobacter chroococcum* B-8 as a function of the concentration of molybdenum or vanadium: Curve I, molybdenum with 2 percent sucrose 2; curve II, molybdenum with 2 percent sucrose 1; curve III, vanadium with 2 percent sucrose 1; curve IV, vanadium with 2 percent sucrose 2;  $\odot$ - $\odot$ , a replot of curve II after correction of total nitrogen for molybdenum impurity (b-a).

with the Warburg technique have indicated some shifting and broadening of this concentration range. *A. vinelandii* V-1 and *A. chroococcum* B-8 respond similarly. As might be expected, a lower concentration of molybdenum is required for the maximum rate of growth of young well-aerated cultures than for the maximum total growth of unaerated long-time Erlenmeyer flask cultures.

Age itself influences the effective suboptimal concentration range of molybdenum, as is shown for *Azotobacter chroococcum* B-8 in figure 6. Although the minimum concentration of molybdenum required to give an effect is about the same for both 1- and 6-day cultures, the optimum concentration for the former is 0.01 or less of that for the latter. The concentration yielding half-maximum growth is likewise shifted from about 0.0013 p. p. m. of molybdenum for the younger cultures to about 0.03 p. p. m. of molybdenum for the older ones.



It has been shown (table 2 and fig. 3) that most strains of *Azotobacter vinelandii* tested were able to fix one-third to two-thirds as much nitrogen in the purest obtainable media as in media with molybdenum added. It might be expected from this that these organisms utilize the molybdenum so much more efficiently that a very low concentration would yield maximum growth, but the tabulation given below shows that this concentration is only slightly if at all lower than the 1 p. p. m. required by *A. chroococcum* B-8. In the light of these data, one might question whether molybdenum should be considered essential for such organisms as *A. vinelandii* B-6. This could be definitely

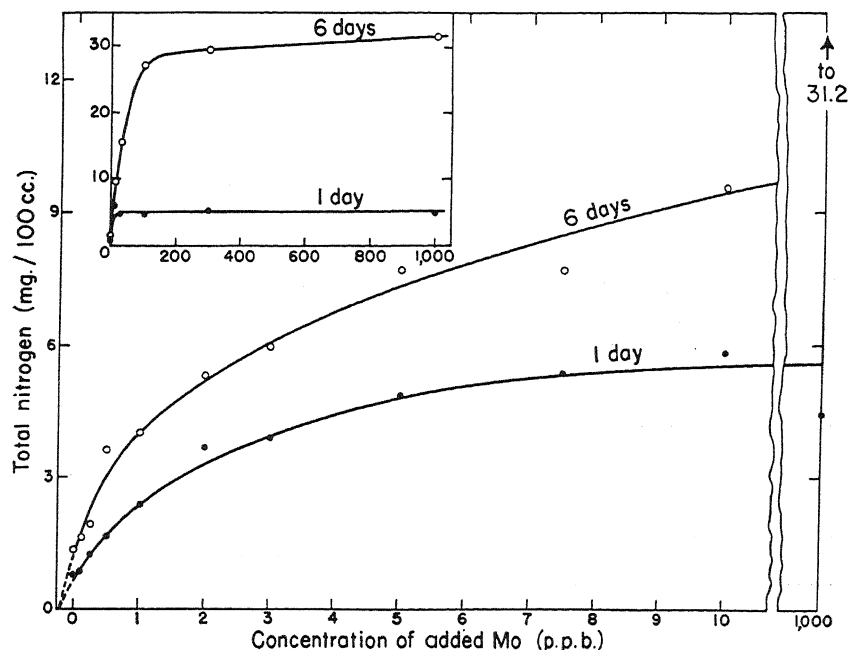


FIGURE 6.—Effect of age upon the effective concentration range of molybdenum for nitrogen fixation by *Azotobacter chroococcum* B-8.

determined, however, only by using media free from all traces of this element.

The effective concentration range of molybdenum for *Azotobacter vinelandii* B-6 (5-day cultures) follows:

Mo added (p. p. m.):	Total N fixed (mg. per 100 cc.)	Mo added (p. p. m.)	Total N fixed (mg. per 100 cc.)
0.0	17.1	0.01	26.4
0.00001	18.4	0.03	29.2
0.00003	17.2	0.1	32.4
0.0001	19.7	0.3	31.6
0.0003	20.5	1.0	32.6
0.001	21.2	10.0	33.1
0.003	24.5		

#### RELATION OF IRON TO MOLYBDENUM EFFECT ON NITROGEN FIXATION BY AZOTOBACTER

Burk, Lineweaver, and Horner (17, 18) showed the importance of humic acids as carriers of iron for *Azotobacter*. Later, Horner and

Burk (23) worked out the effective concentration range of iron for *Azotobacter vinelandii* (0.02–0.5 p. p. m.) and also reported an apparent effect of molybdenum from traces present as impurities in certain iron salts and humus preparations. This effect had previously been thought to be due entirely to iron stimulation. Since the significance of molybdenum in the nutrition of *Azotobacter* was not fully appreciated at that time, the cultures were as a rule suboptimal with respect to molybdenum. It remained for Bortels (11) and Krzemieniewski and Kovats (30) to show that both elements must be present in optimal amounts to produce maximum growth of this organism. Some 5 to 10 times the optimal iron concentration previously reported by the writers was found necessary in the presence of adequate molybdenum. Work at this laboratory has confirmed these findings. Table 3 indicates that any suboptimal concentration of molybdenum allows a certain limit for growth beyond which it is not raised by increasing the concentration of iron. Likewise, with suboptimal iron, increasing concentrations of molybdenum are only partly effective until the concentration of iron is raised. There is also some indication from table 3 that the relative suboptimal concentration effects of molybdenum and iron differ to some extent for *A. vinelandii* V-1 and *A. chroococcum* B-8. Bortel's work also indicates such differences between his strains.

TABLE 3.—Effect of molybdenum in *Azotobacter* cultures supplied with high and low concentrations of iron

[Figures are for 100 cc. of culture]

Trace element supplied		Total nitrogen fixed by—			
		<i>A. vinelandii</i> V-1 (5 days) with—		<i>A. chroococcum</i> B-8 (13 days) with—	
Mo	Fe <sup>1</sup>	Glucose	Sucrose 1	Glucose	Sucrose 1
<i>P. p. m.</i>	<i>P. p. m.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Mg.</i>
0.0002 <sup>2</sup>	0.2	0.51	—	0.98	—
0.0012 <sup>2</sup>	.2	—	2.80	—	3.45
4	.2	5.87	3.01	7.67	10.0
0.0002 <sup>2</sup>	4	.65	—	.85	—
0.0012 <sup>2</sup>	4	—	6.08	—	3.38
4	4	19.6	16.1	22.8	26.7

<sup>1</sup> Iron was supplied as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ .

<sup>2</sup> No molybdenum added, but this concentration of Mo (or V) is estimated to be present in the medium.

Kovats (29) has shown conclusively that different preparations of soil humate may vary markedly in the percentage of available molybdenum. The relatively high concentrations of the humates employed furnished optimum iron in all cases unless they were heated with hydrochloric acid (HCl), which apparently largely dissolved out or inactivated the iron of the humates (or their ash) without significantly affecting their molybdenum content. It is now known that in the early work at this laboratory the medium was deficient in available iron, and the strain of *Azotobacter* employed was one that at times has been observed to fix considerable amounts of nitrogen with mere traces of molybdenum in the medium. It is not surprising, therefore, that the preponderant influence of iron in the humates overshadowed

the effect of molybdenum. Bassalik and Neugebauer (3) have criticized this early work because they were unable to confirm it fully. They obtained greater effects with soil extracts than with a variety of iron compounds and suggested that the molybdenum content accounted for the difference. The quantity of nitrogen fixed by their cultures was too small to permit definite conclusions, but their suggestion is essentially confirmed by the writers' latest work.

Birch-Hirschfeld (5) found that either soil extract or 5 to 250 p. p. m. of molybdenum (0.05 p. p. m. was nearly optimum) gave the same increase in nitrogen fixation by shaken Erlenmeyer flask cultures of *Azotobacter chroococcum*, but that in growth-rate experiments with the Warburg technique only the former was active. The ash of soil extract behaved like molybdenum. The activity of the soil extract on the growth rate was attributed to the organic fraction; and the effect on the ultimate nitrogen fixation, to the molybdenum content. Previous results obtained at this laboratory would indicate, however, that the apparent effect of the organic fraction may be due to the more available iron adsorbed in the organic complex. Ready availability is of course much more important in growth-rate studies than in experiments lasting several days. The failure of molybdenum to stimulate the rate of growth in Birch-Hirschfeld's experiments with the Warburg technique may be attributed to impurity in the medium, which was sufficient for these cultures but not for the heavier Erlenmeyer flask cultures. This is evidenced by the fixation of about 10 mg. of nitrogen per 100 cc. in the absence of added molybdenum or soil extract.

#### RELATION OF OTHER ELEMENTS TO NITROGEN FIXATION BY AZOTOBACTER

The problem of determining whether additional trace elements are required by *Azotobacter* largely resolves itself into a study of strain variation and purity of the media. These factors, which are likely to vary with the investigator, prevent adequate comparisons and generalizations. Schröder (34) has shown the most remarkable insufficiency of molybdenum for several strains of *Azotobacter chroococcum*. When the medium was prepared with distilled water, it was necessary to add molybdenum, zinc, copper, tungsten, and silicon, but when tap water was used the tungsten and silicon could be eliminated without a decrease in growth. Bortels (11) has reported some rather inconsistent results, where at times he found that the addition of manganese or silicon or both was necessary to supplement molybdenum or vanadium for maximum nitrogen fixation; at other times manganese and silicon produced no response. Surprisingly enough, he attributes these variations largely to the effects of weather. Flieg (21), on the other hand, showed with crude cultures of *Azotobacter* that of 18 trace elements added, only the elimination of molybdenum or iron resulted in significantly decreased growth. The absence of molybdenum had less effect than when iron was omitted, probably because vanadium was still present in the medium.

The earlier work at this laboratory to determine the possible substitution of other elements for molybdenum, in which *Azotobacter vinelandii* V-1 and the Warburg technique were chiefly used, gave no indication of replaceability of molybdenum at a concentration of 10 p. p. m. or less by any of 21 elements tested except vanadium.

Bortels (8) has, however, reported some effect of tungsten in certain cases, and Krzemieniewski and Kovats (30) have shown that tungsten in relatively greater concentrations will replace molybdenum to a considerable extent in its effect on *A. chroococcum*.

The results of the writers' recent tests of the effects of manganese and tungsten are given in tables 4, 5, 6, and 7.

Table 4 shows no appreciable effect of 0.01 to 1.0 p. p. m. of manganese in the presence or absence of added molybdenum for three strains, except for an approximately twofold effect on *Azotobacter chroococcum* B-8 without molybdenum. With so little growth this is hardly significant.

TABLE 4.—Effect of manganese on nitrogen fixation by *Azotobacter*

[Duration of experiment, 5 days; energy source, 2 percent sucrose]

Species and strain	Nitrogen fixed with indicated amount of manganese (p. p. m.)						
	Without molybdenum				With 1 p. p. m. of molybdenum		
	0	0.01	0.1	1	0	0.01	1
<i>Azotobacter chroococcum</i> B-8.....	Mg. 1.25	Mg. 1.17	Mg. 1.32	Mg. 2.42	Mg. 26.5	Mg.	Mg. 28.3
Do.....	1.39		1.21				
<i>Azotobacter vinelandii</i> V-1.....	1.21						
<i>Azotobacter vinelandii</i> B-6.....	17.10	18.50		14.80	32.6	34.0	30.8

Table 5 compares the effects of equal concentrations of molybdenum, vanadium, and tungsten on relatively old cultures of two strains. At a concentration practically optimum for molybdenum or vanadium (0.5 p. p. m.) tungsten is seen to have but slight effect on total nitrogen or on ratio of nitrogen fixed to sugar consumed for *A. vinelandii* V-1, and no positive effect for *Azotobacter chroococcum* B-8. Vanadium, on the other hand, at the same concentration gives about 60 to 75 percent of the effect of molybdenum on both growth criteria for the two organisms.

TABLE 5.—Effect of molybdenum, vanadium, and tungsten on nitrogen fixation by *Azotobacter*

Experiment No. and species and strain	Age	Element added		Total nitrogen in 100 cc.	Sugar consumed in 100 cc.	N fixed per gram of sugar consumed
		Symbol	Amount			
Experiment 1:	Days		P. p. m.	Mg.	Gm.	Mg.
<i>Azotobacter vinelandii</i> V-1.....	19			5.4	1.66	3
	19	Mo	0.5	24.5	2.37	10
	19	V	.5	18.1	2.42	7
	19	W <sup>1</sup>	.5	6.8	1.60	4
<i>Azotobacter chroococcum</i> B-8.....	33			4.7	.62	8
	33	Mo	.5	35.8	2.09	17
	33	V	.5	27.6	2.11	13
	33	W <sup>1</sup>	.5	2.7	.35	8
Experiment 2:						
<i>Azotobacter chroococcum</i> B-8.....	19			1.3	.42	3
	19	Mo	1.00	30.8	1.90	16
	19	V	1.00	19.6	1.95	10
	19	Mo	.001	4.4	.71	6
	19	V	.001	3.7	.75	5
	19	( <sup>2</sup> )		4.0	.64	6

<sup>1</sup> Tungsten was added as tungstic acid neutralized with NaOH.

<sup>2</sup> The energy source in this culture was 2 percent of sucrose 1, which supplied 0.0012 p. p. m. of Mo (or V); the remaining cultures of experiment 2 had 2 percent of sucrose 2; all cultures in experiment 1 had 2.5 percent of sucrose 1.



When much higher concentrations of tungsten are employed, as shown in table 6, the growth of *Azotobacter chroococcum* B-8 and C-4 was highly stimulated in the absence of molybdenum but not in its presence. The total nitrogen of the *A. chroococcum* B-8 cultures was increased about 13 times, or to 15.8 mg. per 100 cc., by 50 p. p. m. of tungsten. This is highly indicative of a spurious tungsten effect caused by traces of molybdenum in the tungstic acid employed.

TABLE 6.—Effect of high concentrations of tungsten on nitrogen fixation by *Azotobacter*<sup>1</sup>

Concentration of tungsten (p. p. m.)	Total nitrogen (mg. per 100 cc.)			
	<i>A. chroococcum</i> B-8		<i>A. chroococcum</i> C-4	
	No Mo	Mo (1 p. p. m.)	No Mo	Mo (1 p. p. m.)
0.....	1.22	25.0	0.86	15.5
1.....	2.85	26.9	1.60	14.9
10.....	8.85	26.1	-----	-----
50.....	15.80	25.0	3.87	10.0

<sup>1</sup> Cultures analyzed after 6 days; energy source, 2 percent of sucrose 3; tungsten added as tungstic acid neutralized with NaOH.

Table 7 gives the results of an experiment in which four different compounds of tungsten were tested, at several concentrations up to 250 p. p. m. of tungsten, for their effect on nitrogen fixation by the two strains of *Azotobacter chroococcum*. One of these compounds is a sodium tungstate salt that had been commercially purified by the Folin method to remove molybdenum impurities. It is seen that this salt, even in a concentration of 250 p. p. m. of tungsten, caused no significant improvement in the growth of either strain, whereas the other three compounds all gave variable but marked stimulation; the potassium tungstate in concentrations of 10 to 50 p. p. m. of tungsten yielded the maximum growth normally obtained with 1 p. p. m. of molybdenum. Quantitative chemical analyses of these tungsten compounds for molybdenum by the Folin and Trimble method (22) gave the following results: Sodium tungstate ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) (Folin), negative ( $<0.001$  percent);  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , 0.014 percent; tungstic acid ( $\text{H}_2\text{WO}_4$ ), 0.034 percent; and potassium tungstate  $\text{K}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ , 0.125 percent. The indicated impurity in the Folin-purified salt may well have been considerably less than the minimum given. The limit of the test was not accurately determined, but this compound gave an absolutely negative test on a sample several times greater than those of the other compounds. From the analyses of the different compounds just given, the amount of molybdenum furnished by each concentration of tungsten added was estimated and is given in table 7. The amount of nitrogen fixation obtained with the two strains is nearly equal to but slightly higher than that to be expected from the quantities of molybdenum impurity added. Hence, the chief effect from the tungsten compounds is undoubtedly due to their molybdenum impurities. These results also strongly indicate that the marked stimulation caused by sodium tungstate of nitrogen fixation by *A. chroococcum*, reported by Krzemieniewski and Kovats (30), was the result of molybdenum impurity.

TABLE 7.—Effect of tungsten compounds containing various amounts of molybdenum as impurity on nitrogen fixation by *Azotobacter*

[Energy source, 2 percent of sucrose 3]

Tungsten added		Mo added as impurity	Mo added as molybdate	Total nitrogen per 100 cc.	
Source	Quantity of W			<i>A. chroococcum</i> B-8 <sup>1</sup>	<i>A. chroococcum</i> C-4 <sup>2</sup>
	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>Mg.</i>	<i>Mg.</i>
None.....	0			1.31	1.30
	0		1	32.1	29.7
	1	<0.00002		1.20	1.22
	10	<.0002		1.37	1.27
	50	<.001		1.37	1.36
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O (Folin <sup>3</sup> ).....	50	<.001	1	29.0	26.4
	250	<.005		1.27	1.15
	1	.0003		2.49	2.05
	10	.003		14.1	4.29
	50	.015	1	33.7	11.3
Na <sub>2</sub> WO <sub>4</sub> ·2H <sub>2</sub> O.....	50	.075		17.2	22.9
	250	.0005		2.89	2.03
	1	.005		8.81	7.50
	10	.025		19.3	24.8
	50	.025	1	28.7	28.9
H <sub>2</sub> WO <sub>4</sub> (neutralized).....	250	.125		25.2	30.5
	1	.0025		8.72	5.34
	10	.025		25.8	26.2
	50	.125	1	30.7	28.0
	250	.625		29.1	29.0

<sup>1</sup> Analyzed after 6 days.<sup>2</sup> Analyzed after 8 days.<sup>3</sup> This compound of tungsten is a commercially purified salt prepared according to the method of Folin to remove molybdenum impurities. The other compounds are customary c. p. products.

## SUMMARY

The influence of molybdenum and vanadium on nitrogen fixation by several strains of *Azotobacter* grown in nonaerated solution cultures was studied.

Eight strains of *Azotobacter chroococcum* and one of *A. vinelandii* showed a tenfold to thirtyfold increase in nitrogen fixation following additions of optimum molybdenum. Two strains of *A. agile* and three of *A. vinelandii* showed only a twofold effect of molybdenum because of a larger fixation in its absence. Maximum nitrogen fixation with optimum molybdenum was, with few exceptions, of the same order of magnitude for all strains tested. Usually this element slightly increased the nitrogen content of the cells.

The quantity of nitrogen fixed per unit of carbohydrate consumed was usually twofold to threefold greater for the *Azotobacter chroococcum* cultures following additions of molybdenum; with the other organisms efficiency was improved to a lesser extent. In the *A. chroococcum* cultures not supplied with molybdenum very little more nitrogen was fixed after an incubation period of 31 days than in 1 week, and usually 50 to 75 percent of the sugar supplied remained unconsumed.

Various samples of sugars supplied about 0.0002 to 0.001 p. p. m. of molybdenum or vanadium impurity when added to media at a concentration of 2 percent. This impurity could be partly removed by filtering through charcoal or by recrystallization from alcohol.

The effective concentration range for molybdenum and vanadium was found to vary from 0.00001 or 0.0001 p. p. m., which gave a detect-

able effect with *Azotobacter chroococcum*, to about 1 p. p. m., which gave maximum growth in 6 days. With younger cultures the range was appreciably narrower, 0.005-0.01 p. p. m. giving maximum growth for 1-day cultures. The concentration range for vanadium was approximately the same as for molybdenum, but the maximum effect was only 50 to 80 percent of that produced by molybdenum.

Experiments dealing with the relation of other elements to molybdenum confirmed the work of previous investigators in showing that both iron and molybdenum must be present in optimum concentrations for maximum growth and fixation. Manganese had no appreciable effect on *Azotobacter* in the presence or absence of molybdenum. Relatively high concentrations of unpurified tungsten compounds were effective as partial substitutes for molybdenum, but this effect could be correlated with the amount of molybdenum present as impurity in these materials.

The essentiality of molybdenum or vanadium for nitrogen fixation by many strains of *Azotobacter* seems to be established by the experiments reported here. Its essentiality for other strains that fix considerable nitrogen in media containing only traces of these elements is, of course, still open to question.

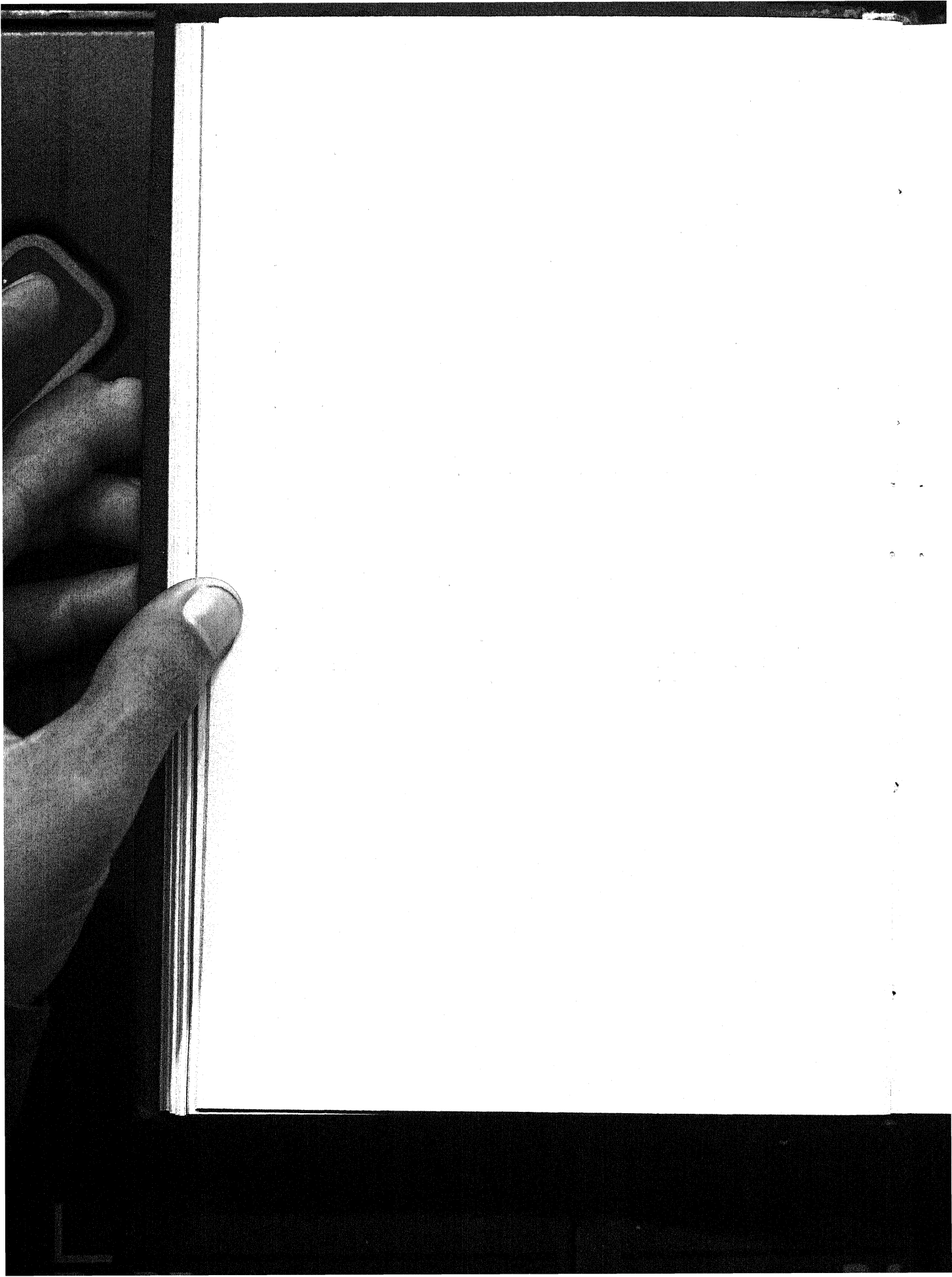
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# EFFECTS OF SOME MILD FORMS OF MOSAIC ON POTATO AND A FEW OTHER PLANTS<sup>1</sup>

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## INTRODUCTION AND HISTORICAL REVIEW

In nearly all work done on the many different types of mosaic attacking a variety of hosts the more devastating types of mosaic and the hosts of greater commercial importance have commanded most attention.

The reports of the many investigators of the effects of mosaic on the leaves vary with the hosts and types of mosaic. While Smith (16)<sup>2</sup> found the yellow parts of mosaic leaves of *Datura stramonium* thicker than healthy leaves, in *Phytolacca decandra* she found the yellowed leaves to be thinner. Schaffnit and Müller (15) report mild mosaic spots thinner than normal green parts. Melchers (14), working with potato, Iwanowski (12), with tobacco, and Dickson (5) found the proportion of thickness in light and dark areas approximately 2:3. Although Woods (22) makes no numerical comparison of leaf thickness he describes the diseased tissues of the tobacco leaf as consisting of closely packed spongy parenchyma, probably resulting in decreased leaf thickness. According to Cook (4), different parts of the same healthy leaf vary in thickness; and comparisons of leaf thickness must therefore be based on many measurements and expressed in very general terms. Certainly where the palisade is much shortened, cuboidal, or undifferentiated and the intercellular spaces are smaller than normal, there can be little doubt as to the decreased thickness of the yellow parts of mosaic leaves (1). Probably all deviations from normal in the yellow parts of leaves should not be ascribed to virus. In rugose mosaic, and at times in mild mosaic, the green parts of affected leaves are thicker than the healthy leaves (16).

Again, in the matter of size and number of chloroplasts and amount of starch present authors disagree. Cook (3) found in sugarcane and tobacco, Westerdijk (21) in tomato, Smith (16) in *Datura stramonium*, and Hoggan (11) in a number of solanaceous plants a reduction in both size and number of chloroplasts in areas affected by mosaic. On the other hand, Dickson (5), Woods (23), Melchers (14), Iwanowski (12), and Smith (16) noted in various plants a reduction in the number but not in the size of chloroplasts in affected leaves. These infections were probably light, for Dickson (5) states that reduction in number precedes reduction in size, and slightly affected plastids show reduction only in chlorophyll. Yet Smith (17) mentions reduction only in number of plastids in cells so seriously affected that walls were ruptured and nuclei were disintegrating.

To complete the series of possible variations in chloroplast response to the presence of virus, Doolittle (6), working with cucumber, Cook (4) with tobacco and tomato, and Clinch (2) with potato, found a

<sup>1</sup> Received for publication September 30, 1941.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 206.

reduction in size of plastids but not in number. Melchers (14) found no difference in number or size of chloroplasts in tomato.

Some investigators believe that the chloroplasts in mosaic leaves gradually degenerate and disintegrate. K  ning (13) found this to be true in tobacco, Dickson (5) and Eckerson (8) in tomato, Sorokin (19) in tomato, and Hoggan (11) in potato in very severe cases. Hoggan (11) admits degeneration of plastids only in extreme infections, and Dickson (5) mentions degeneration as one of the several effects of mosaic on leaves. Cook (4), however, thinks that any degeneration of plastids which others may have observed should be attributed to old age or to causes other than the mosaic virus. Goss and Peltier (10) believe that degeneration is not consistent with the recovery or masking so generally observed at 20   C. and above, for which Cook (4) says increase in size and number of chloroplasts is responsible.

Iwanowski (12) found chloroplasts in mosaic tobacco well filled with starch, as did Clinch (2) in the potato. Westerdijk (21) found little starch in yellowish plastids of tomato. Dunlap (7) found 40 percent as much starch in mosaic tobacco as in healthy tobacco, with small difference between the yellow and green regions of the former. In mosaic leaves starch was more readily converted into simple compounds. Goldstein (9) found no starch in plastids of mosaic tobacco. Stone (20) determined the lower carbon-fixing and starch-storing ability of mosaic-infected potatoes.

The nucleus is often mentioned in connection with the bodies sometimes associated with the presence of mosaic virus, but the fact that few references have been made to variations in nuclei in mosaic leaves probably indicates that they are not so readily affected as chloroplasts. Cook (3) examined nuclei of sugarcane and tobacco and reported no reduction from normal.

#### METHODS

Study was concentrated for the most part on an important host, the potato (*Solanum tuberosum* L.), but the mosaic infecting it was a very mild and relatively harmless type. Emphasis was placed on the response of the plants to the virus rather than on the identification of the virus. *Solanum* virus 1 was probably present in both the diseased and the so-called healthy potato plants since, according to Smith (18), it is almost universally present in the commercial stocks of America though not always apparent. The virus, which in combination with *Solanum* virus 1, produced the symptoms seen in the diseased plants, may very well have been *Solanum* virus 3. Groundcherry (*Physalis* sp.), raspberry, and blackberry (*Rubus* spp.) infected with mosaic were studied in less detail. No attempt was made to identify the viruses concerned in these plants. The infections are referred to as mild forms in a general sense.

Plants for the investigation were obtained from several sources. The field of a market gardener furnished several mosaic Early Rose potato plants, and another field supplied many mosaic Green Mountain plants. The other mosaic and healthy plants were grown from seed potatoes obtained in the market, some local and uncertified, some from Maine. Healthy plants were obtained from the latter source only. Successive plantings were made beginning in early June. At all times healthy and mosaic plants used for examination or for fixation were



grown out of doors and matched as nearly as possible in age, water supply, light supply, cultivation, and variety.

Fixed and stained material was used for the most part since many of the comparisons to be made were favored by uniform thickness of sections, sharpness of detail brought out only by staining, and the use of pieces too small to be satisfactorily handled as freehand sections.

Fixations were made with Flemming's medium and Allen's B15 solution. A fast green-safranin stain was used in most of the work, but was supplemented by iron haematoxylin in some cases. Fast green-safranin gives good contrast in a healthy cell, chloroplasts appearing brilliant red, cytoplasm pink, nuclei a greenish blue with red nucleoli, and walls a sharp green. The starch does not stain so the grains appear as white spots in the chloroplasts.

Depending on the size of the tissue or organ to be examined, drawn, or measured, 20 $\times$  or 15 $\times$  eyepieces and 40 $\times$  or 60 $\times$  objectives were used. Drawings were made with the aid of a camera lucida and measurements were taken with a calibrated, movable-scale eyepiece micrometer.

## EFFECT OF MOSAIC ON POTATO

### LEAF MEASUREMENTS

As stated, the mosaic was a mild type, and it was to be expected that the effects would be correspondingly slight. A series of measurements was made on one set of leaves, taken from the same diseased and the same healthy plants all of one variety (upper section, table 1). Although a decrease in thickness in diseased leaves is progressive, the amount is slight and does not approach the 2:3 ratio which is said to occur in more severe types of mosaic.

TABLE 1.—*Thickness of leaves and palisade layers in mosaic and healthy plants of one variety*

### AVERAGE ON FIRST SET OF LEAVES

Plant	Leaf thickness	Palisade	
		Thickness	Total leaf thickness
	<i>Microns</i>	<i>Microns</i>	<i>Percent</i>
Mosaic:			
Yellow part of leaf.....	187	62	33
Green part of leaf.....	190	68	36
Healthy.....	197	68	35

### COMBINED AVERAGE ON FIRST SET AND LEAVES FROM RANDOM PLANTS

Mosaic:			
Yellow part of leaf.....	175	58	33
Green part of leaf.....	181	64	35
Healthy.....	190	67	35

The measurements on the first set of leaves were averaged with measurements on leaves from 7 other plants taken more or less at random, making a total of over 300 measurements (see lower section, table 1). The paler parts of the mosaic leaves were only 8 percent thinner than similar healthy leaves and only 3 percent thinner than adjoining green parts. In those leaves that were most nearly matched the pale parts were only 5 percent thinner than the healthy leaves and

1.5 percent thinner than the adjoining green parts. Figure 1 shows an exceptionally pronounced example of decrease in leaf and palisade thickness in yellow parts of mosaic potato leaf.

The palisade cells in mosaic leaves appear to have developed to their normal length. The 2 percent difference in proportion of total leaf thickness occupied by the palisade layer in mosaic yellow and normal leaves certainly is not striking.

Groups of mesophyll cells from nine mosaic and healthy leaves were drawn with the aid of a camera lucida and the amount of intercellular space in a given area was determined by a planimeter as follows: Yellow mosaic, 16 percent; green mosaic, 13 percent; healthy, 11



FIGURE 1.—Sections of potato leaves: A, healthy; B, mosaic. ( $\times 550$ ).

percent. In general, the sections of healthy leaf mesophyll showed a smaller area in intercellular spaces than did those of diseased leaves.

#### SIZE AND NUMBER OF CHLOROPLASTS

Since mosaic is evident as a variation in the amount or shade of green in a leaf it is logical to expect some microscopic differences in the chloroplasts of leaves attacked by mosaic. The pale color of mottled leaves obviously may be the result of one or more conditions: viz, a subnormal amount of chlorophyll in each plastid, smaller plastids, or fewer plastids. No attempt was made in this study to determine the comparative amounts of chlorophyll in the plastids of pale and green leaf parts.

The tendency of normal plastids is to occupy all available space along the wall surface of mature cells so completely as to appear angular from mutual pressure (fig. 2, A). Plastids of diseased cells, being not only smaller but also less numerous, are so distributed as to appear rounded (fig. 3).

Samples were taken of a series of mature leaves from a plant in which the yellowing caused by mosaic became progressively more pronounced. Samples from matched leaves of a healthy plant were fixed and stained under the same conditions. Table 2 gives the results of the measurement of chloroplasts from these leaves.

TABLE 2.—*Size of chloroplasts in a series of healthy and mosaic leaves showing simultaneous increase in degree of yellowing and age*

Leaves of increasing age	Average diameter of chloroplast	Comparison of chloroplast size
	Microns	Percent
Slight yellowing:		
Yellow part.....	3.73	9.7 less than healthy.
Green part.....	3.97	3.9 less than healthy.
Healthy.....	4.13	
Pronounced yellowing:		
Yellow part.....	2.89	35 less than healthy.
Green part.....	3.39	24 less than healthy.
Healthy.....	4.48	
Severe yellowing:		
Yellow part.....	1.47	62 less than healthy.
Green part.....	2.12	45 less than healthy.
Healthy.....	3.87	6 less than youngest healthy leaf.

The 6 percent decrease in the size of the healthy leaf chloroplast indicates a gradual, slight effect of age on the plastid. However, the size of the plastids in the yellow and green parts of mosaic leaves decreased 61 and 47 percent respectively. No doubt age takes a heavier toll on chloroplasts that are already weakened by mosaic. The chloroplasts of the green parts of the mosaic leaves are more susceptible to aging than those of a healthy leaf. The difference in size of healthy and diseased plastids is noticeable in all the accompanying text figures.

Chloroplasts applied to cell walls were measured in mosaic and healthy potato and tomato leaves. Table 3 shows the length and width of these chloroplasts.

TABLE 3.—*Size of elongated chloroplasts in cells of healthy and mosaic leaves*

Plant	Length of chloroplast	Width of chloroplast	Plant	Length of chloroplast	Width of chloroplast
Potato:	Microns	Microns	Tomato:	Microns	Microns
Mosaic.....	3.75	1.69	Mosaic.....	5.13	1.98
Healthy.....	4.06	1.89	Healthy.....	5.80	2.43

There are several ways of stating the number of plastids in diseased and healthy cells. A set of six plants, mosaic and healthy, was used for one determination of plastid number per cell. Only palisade cells were drawn, together with their plastids. The average number of plastids per cell was as follows: In yellow parts, 41; in green parts, 47; and in healthy leaves, 53; that is, the palisade cells of the yellow parts contained about 13 percent fewer plastids than the adjoining green parts and 23 percent fewer than healthy leaves. Care was taken to match healthy and diseased plants both in age and environment. The differences appearing in size and number of plastids in this set of plants may therefore be considered significant.

Another manner of expressing and comparing plastid numbers is in terms of a unit of area. The areas of a block of cells drawn with the aid of a camera lucida were measured by means of a planimeter. The plastids in these areas were counted and the number of plastids per 100 cm.<sup>2</sup> of cell area as it appeared on the drawing paper was computed. Eight samples gave the following results:

Mosaic potato:	Plastids per 100 sq. cm.
Yellow part of leaf.....	238
Green part of leaf.....	225
Healthy potato.....	192

In the process of fixation the palisade cells of diseased leaf sections seemed to contract somewhat so that the edges of these cells were

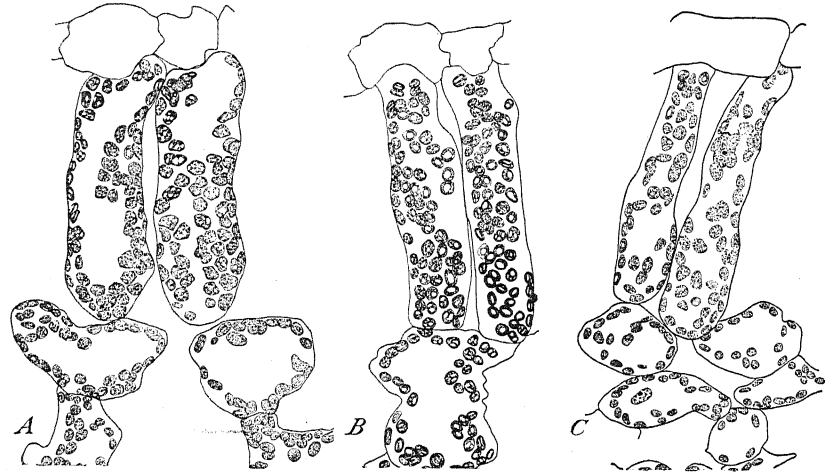


FIGURE 2.—Sections of healthy potato leaf showing condition of chloroplasts and starch: A, at 9 a. m.; B, at 4 p. m.; and C, after 20 hours of darkness.  $\times 500$ .

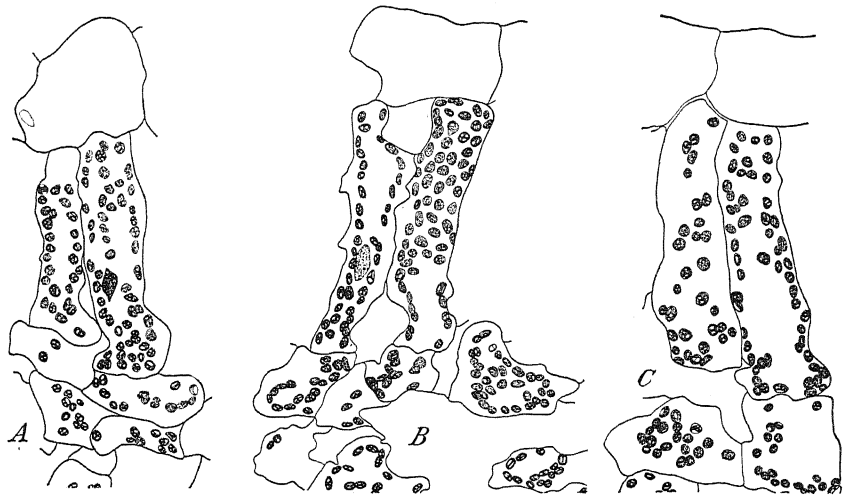


FIGURE 3.—Sections of yellow part of mosaic potato leaf showing condition of chloroplasts and starch: A, At 9 a. m.; B, at 4 p. m.; and C, after 20 hours of darkness.  $8\times 500$ .

fluted. This condition might affect the area of the palisade cells, but it would not influence the total number of plastids within each cell. However, it might account for the seemingly high plastid count in unit areas of diseased leaves.

Still a third way of comparing plastid quantity is in terms of area of plastids. If diseased plastids are much smaller than healthy ones,



they will furnish less working capacity even though they are more numerous. Table 4 gives a comparison of plastid size and area of plastids per 100 cm.<sup>2</sup> in all samples taken, drawn with camera lucida at 1660 $\times$  magnification.

TABLE 4.—*Comparison of plastid area in a given area of leaf cells of mosaic and healthy potatoes*

Plant	Chloroplasts			
	Diameter	Area	Per 100 cm. <sup>2</sup> of cells	Area per 100 cm. <sup>2</sup> of cells
Mosaic:	<i>Microns</i>	<i>Square microns</i>	<i>Number</i>	<i>Square microns</i>
Yellow part of leaf.....	2.8	6.2	225	1,395
Green part of leaf.....	3.2	8.0	208	1,664
Healthy.....	3.9	11.9	201	2,392

Though by this criterion the number of plastids in a given area is 12 percent greater in the yellow parts, individually they are only about half as large in area in the yellow parts, with a total area only 58 percent as great as that of the plastids in a corresponding area of healthy leaf tissue. This situation is brought out in figures 2 to 5.

There are two striking differences between mosaic-infected and healthy plastids as seen in a stained section: (1) The smaller diameter of the mosaic plastids, especially in the yellow parts; and (2) the reaction of mosaic plastids to the safranin stain (fig. 5). Healthy plastids stain a brilliant red while those in yellow areas of mosaic leaves and, to a certain extent, those in adjoining green areas do not retain the safranin and so merge into the surrounding haze of cytoplasm.

#### CARBOHYDRATE FIXATION BY HEALTHY AND MOSAIC-AFFECTED CHLOROPLASTS

If the presence of mosaic virus in the cell does not appreciably change the microscopic appearance of an individual plastid aside from its size, its effect may be apparent in the amount of starch produced by the plastid.

Corresponding leaves on similar mosaic and healthy plants were chosen to furnish material for study of starch production. In the forenoon a green and a yellow piece were cut from a mottled leaf and a piece from the healthy leaf. These were fixed in the same vial, being recognized by shape. In the afternoon a similar set of pieces was taken from the same leaves. The plants were then closely covered to exclude light until the time of the third sampling, the next forenoon. The three sets of leaf pieces were run through the process of fixing and embedding simultaneously and mounted in sets made up either of yellow, green, and healthy pieces at a given time or of yellow, green, or healthy pieces at the three different hours. This procedure prevented variation due to unequal staining and destaining. The fast green safranin stain was used.

At 9 a. m. healthy chloroplasts contained many small starch grains (fig. 2, A). At 4 p. m. the starch grains appeared to be much less numerous but were larger; in fact, often a single starch grain so filled the chloroplast that the chloroplast itself appeared as a heavy ring around the starch grain or as a rim on one side of the grain (fig. 2, B). After about 20 hours of darkness, during which

the plants were covered, much of the starch had disappeared from the healthy plastids, some appearing entirely devoid of starch (fig. 2, *C*)

At all hours of sampling the small yellow plastids seemed to contain about the same amount of starch (fig. 3). They were never filled to



FIGURE 4.—Sections of green part of mosaic potato leaf showing condition of chloroplasts and starch: *A*, At 9 a. m.; *B*, at 4 p. m.; *C* after 20 hours of darkness.  $\times 500$ .

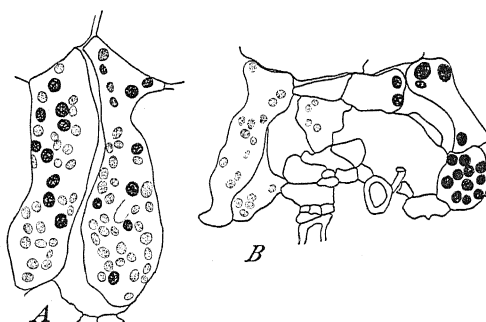


FIGURE 5.—*A*, Cells from green area of mosaic potato leaf showing two sorts of chloroplasts in same cell; *B*, cells from yellow area (left) and adjoining green area (right).  $\times 500$ .

capacity. Starch grains tended to be small and fairly numerous. The slight differences followed the characteristics of the healthy leaf; i. e., the plastids in the 4 p. m. samples contained the most starch of the three and the plastids in the sample taken after the plant was covered contained the least. Translocation seems not to have gone so far in the mosaic plastids as in the healthy ones. Green parts of mosaic leaves behave much more like yellow parts of the same leaves than like healthy leaves. Starch grains are small and numerous, never filling the plastid, and translocation appears to be even slower than in the yellow parts (fig. 4).

## SIZE OF NUCLEUS

Although the nucleus will usually withstand unfavorable conditions as long as any organ of the cell it was thought best to make a series of measurements. Since round nuclei are uncommon, two measurements were taken of each nucleus, length and width, and sizes are so expressed.

The nuclei of cells in the green and yellow parts of mosaic leaves differ little in size, those in the green parts tending to be slightly larger. The nuclei of healthy leaves, however, are considerably larger, being 42 percent wider and 55 percent longer than the nuclei of the yellow parts of mosaic leaves. The upper part of table 5 is made up of about 325 measurements. These were combined with 270 more and the total, approximately 600 measurements, is given in the lower part of table 5.

TABLE 5.—Average size of nuclei in cells of yellow mosaic, green mosaic, and healthy leaves in a series of 9 matched leaf pieces

BASED ON ABOUT 325 MEASUREMENTS

Plant	Width of nucleus	Length of nucleus
	<i>Microns</i>	<i>Microns</i>
Mosaic:		
Yellow part of leaf.....	4.5	6.7
Green part of leaf.....	5.0	6.8
Healthy.....	6.4	10.4

BASED ON ABOUT 600 MEASUREMENTS

Mosaic:		
Yellow part of leaf.....	4.8	7.1
Green part of leaf.....	4.6	7.3
Healthy.....	6.1	9.6

Table 5 seems to afford sufficient evidence to justify the conclusion that the nuclei of healthy leaves are larger than those of diseased leaves whether green or yellow. Nuclei in healthy and mosaic leaves of corresponding ages were measured at different intervals, but there was no indication of a decrease in size with increasing age. In fact, for the most part the nuclei of older leaves were slightly larger (table 6).

TABLE 6.—Size of nuclei in leaves of increasing age, 1 indicating youngest leaf

Condition and age of leaf	Width of nucleus	Length of nucleus
	<i>Microns</i>	<i>Microns</i>
Yellow:		
1.....	4.18	6.64
2.....	4.45	6.80
3.....	4.73	6.68
Green:		
1.....	4.10	6.93
2.....	5.32	7.03
3.....	4.52	6.28
Healthy:		
1.....	6.82	9.91
2.....	5.92	10.31
3.....	6.40	11.06

EFFECT OF MOSAIC ON RASPBERRY, BLACKBERRY, AND  
GROUNDCHERRY

Though the potato has taken the leading role in this series of studies, a few other plants were also used. For epidermal studies material was taken from raspberry, blackberry, and groundcherry (*Physalis*). The mosaics, mild in form, were unidentified.

The mosaic raspberry leaves were strikingly mottled but the surfaces were smooth. Since the lower epidermis of raspberry is hairy and very difficult to detach from the mesophyll only the upper epidermis was examined. The upper epidermis may be more affected than the lower or it may indicate directly the effect of the mosaic on the lower epidermis. However, many groups of about 30 epidermal cells each were drawn with camera lucida, 675 $\times$  magnification, and the area of the groups determined by means of a planimeter. The results show the area of the upper epidermal cells to be as follows: Mosaic plant, yellow part of leaf, 256 square microns; mosaic plant, green part of leaf, 404 square microns; leaves from healthy plant, 575 square microns.

Both the upper and lower epidermis of blackberry leaves affected by mosaic of two types were measured. Mosaic leaves of the rugose type showed a marked humping of the green areas, the yellow areas remaining smooth. In general the difference in size of the epidermal cells followed the expected course. The lower epidermal cells of the concave surfaces were smaller than the cells on the smooth surfaces, but not significantly so. In the upper epidermis, however, the cells along the contours of the convex surfaces were much larger than those of the adjoining smooth surface. The variation in size in these cells may therefore be considered as due primarily to the contours of the leaf surface, one of the results of the presence of mosaic.

TABLE 7.—Comparison of epidermal and guard cells in mosaic and healthy blackberry leaves

RUGOSE MOSAIC					
Leaf	Area			Stomata per 100 lower epidermal cells	Stomata per 1,000 $\mu^2$ of epidermal cells
	Epidermal cells		Stomata		
	Upper	Lower			
	<i>Square microns</i>	<i>Square microns</i>	<i>Square microns</i>	<i>Number</i>	<i>Number</i>
Mosaic:					
Yellow, smooth.....	640	247	235	24	0.97
Dark green, puffed.....	1,163	224	241	32	1.40
Healthy.....	660	342	261	33	.96
MOTTLED MOSAIC					
Mosaic:					
Yellow.....	458	154	210	20	1.30
Green.....	544	179	190	16	.88

Differences in size of the guard cells in green and yellow areas were negligible (table 7, upper section). No stomata were found on the upper surface of either mosaic or healthy leaves. The number of stomata per 100 epidermal cells was found to be reduced, but in terms of number per unit of area, which, after all, is more important



from the standpoint of their use to the plant, the yellow areas were fully as well equipped as the normal. Again the squeezing together of the cells on the concave lower surface of the green parts of the mosaic leaf accounts, in part at least, for the increased number of stomata in these areas. The reduction in number of stomata in the yellow areas may be directly due to the disease. Decrease in number of stomata might further curtail the production of starch by reducing the amount of carbon dioxide available to diseased plastids.

Leaves of a second type of mosaic blackberry were mottled but smooth throughout. Thus any differences in size of cells may logically be attributed to the mosaic virus. Here only mosaic-affected leaves were used. The lower section of table 7 shows the results of the measurements.

For the study of groundcherry both normal and mosaic leaves were available. The dark green parts of mosaic leaves were only slightly convex, if at all, so any deviations from normal or from yellow areas may be directly related to the disease.

The number of stomata was affected in both green and pale areas, though to a much greater degree in the pale areas (table 8). Here, again, the size of the guard cells remained nearly constant, showing only a four percent decrease in the yellow area. The epidermal cells, on the other hand, showed an average decrease in size of 55 percent in the mosaic leaf as a whole. The decrease in the size of the epidermal cells in the green areas of the mosaic is surprisingly high.

TABLE 8.—*Comparison of epidermal and guard cells in mosaic and healthy groundcherry leaves*

Leaf	Areas in—			Stomata per 100 lower epidermal cells	Stomata per 1,000 $\mu^2$ of epi- dermal cells
	Epidermal cells		Stomata		
	Upper	Lower			
	<i>Square microns</i>	<i>Square microns</i>	<i>Square microns</i>	<i>Number</i>	<i>Number</i>
Mosaic:					
Pale green.....	623	404	355	13	0.3
Dark green.....	632	458	357	23	.5
Healthy.....	1,500	815	369	32	.4

#### SUMMARY

Width of the palisade layer and thickness of leaf in potatoes are little affected by mild mosaic virus.

In general, intercellular spaces in the diseased leaves of potato are larger than in healthy leaves.

Chloroplasts are reduced in size and number in potato leaves that show mild mosaic mottle.

Chloroplasts in affected areas can be readily distinguished from healthy chloroplasts by their staining reaction.

In chloroplasts of mottled leaves, starch grains are smaller and more numerous than in chloroplasts of healthy leaves. This starch is not readily removed from the plastids during hours of darkness.

The nuclei of mottled potato leaves are considerably smaller than those of comparable healthy leaves.

Cells in the upper epidermis of yellow parts of mosaic raspberry leaves are appreciably smaller than those of either green parts of the same leaves or of healthy leaves.

Blackberry leaves affected by rugose mosaic show upper epidermal cells in green parts larger than in yellow parts and lower epidermal cells smaller than in yellow parts.

Comparison of size and number of stomata in green and yellow parts of the rugose mosaic blackberry leaf shows no consistent difference.

In blackberry affected with mild mosaic, epidermal cells are smaller in yellow parts than in green, but stomata are larger and more numerous.

In groundcherry, epidermal cells are considerably larger in healthy leaves than in mosaic leaves, but stomata vary little in size or number, at least on an area basis.

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## VARIABILITY OF ERODED MATERIAL<sup>1</sup>

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### INTRODUCTION

Soils that are subjected to sheet erosion are modified progressively. Productivity is decreased, and measurable changes may occur in the chemical and physical properties of the eroding soil. The changes are due in part to the appearance of subsoil in the zone of tillage. Other soil changes may result from a selective removal of the finer soil fractions by the erosion process. Consideration of the latter point has led to speculation as to the relative severity of erosion losses in relation to quality as well as quantity, and some investigations on the variability of eroded materials have been made.

Middleton, Slater, and Byers (6)<sup>2</sup> analyzed mechanically the annual soil losses from various station erosion plots. They found that the texture of the eroded material was finer in some cases than that of the corresponding soil and that it varied with the quantity. In practically every case the material was shown to be appreciably higher than the plot soils in organic content, but in four soils out of eight the mechanical composition of the soil and its eroded material were much the same. These results, therefore, were somewhat at variance with the fact that soil organic matter tends to be concentrated in the finer fractions. The authors of the paper held that their data were insufficient to justify final conclusions.

A more complete study of the mechanical composition of soil losses has been made by Diseker and Yoder (3), using the aggregate analysis method of one of the authors. They concluded that—

In general, soil material is moved layer by layer in the sheet erosion process. The relative loss of colloidal material may be excessive under a condition or combination of conditions which results in small quantities of runoff or in runoff of low velocity or both.

Organic-matter content was not reported.

Rogers (8) has reported on chemical variations in eroded materials in a recent study of plant nutrient losses from the Dunmore silt loam. Studies of bacteriological variability have been made by Wilson and Schubert (10) and by Norman and Newman (7).

The present study was started to clarify certain interrelationships in the variability of eroded material and, more specifically, to determine why organic-matter content fails to follow textural analysis. The point is important, for otherwise textural analysis should govern to a marked degree the chemical and physical properties of the material, insofar as they differ from those of the parent soil.

<sup>1</sup> Received for publication February 25, 1942. Cooperative investigations of the Office of Research, Soil Conservation Service, U. S. Department of Agriculture, and the New York State Agricultural Experiment Station, Geneva, N. Y.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 218.

This is evident, in part, from a consideration of the inert character of sands and silts. The difficulties encountered in former attempts to fractionate soil colloids (2) complete the evidence by demonstrating indirectly the inadequacy of rainfall or runoff as agents to fractionate the finest soil fractions. Inclusion of organic matter or excessive fines in the eroded material causes it to be different from the soil in other measurable properties, such as rate of carbon dioxide production, or its content of exchangeable bases, but the differences are almost certainly due to the mechanical segregation that has taken place. Except for organic matter, chemical and textural analyses are in effect alternate measurements within the same range of variability. It appears therefore that if the chemical properties of the finer soil separates are known, the variability of erosion losses may be judged solely by the nature of their organic content and textural composition.<sup>3</sup> General chemical examinations have been omitted from the present study.

#### EXPERIMENTAL METHODS

Five plots were available from which eroded material was collected and analyzed. These were 1/100-acre plots, with metal boundaries, 6 feet wide and 72.6 feet long. The plots are fitted with devices to intercept and collect all soil and water lost by runoff.

The plots are located on Ontario loam, a relatively permeable soil. Plots 1 and 3 were in red clover (*Trifolium pratense*) and alsike clover (*T. hybridum*), respectively, during the course of the experiment. Plot 2 is planted to winter rye (*Secale cereale*) that is turned under as green manure, and has sufficient cultivation to maintain a summer fallow free from vegetation. Plot 4 is planted to soybeans (*Soja max*) annually. Plot 5 is a spaded fallow, with periodic cultivation to destroy vegetal growth.

Sampling of eroded material was accomplished by passing the runoff from each rain through a divider designed<sup>4</sup> for this purpose by Uhland and Woodruff. The use of the divider entailed an undue amount of labor when a large runoff was encountered, but it was entirely satisfactory for the smaller amounts that were usual under local conditions. Preliminary experiments had shown that the divider gave reliable sampling of local soil losses.

Mechanical analyses were made by the pipette method. In the studies on organic matter some mechanical separations were made by wet sieving after dispersion. A combustion furnace was used to determine total carbon, and organic matter was then estimated through the use of the conventional factor, 1.724.

Samples of the plot soils that were analyzed for comparison were obtained at random. Six samples were taken from each plot.

#### THE DATA

##### ANALYSIS OF PLOT SOILS AND ERODED MATERIAL

Analyses of the eroded material are based on composite samples that are representative of a year's erosion. The data, together with the textural analyses of the plot soils, expressed as averages of six random samples, are shown in table 1. Organic-matter percentages also are recorded.

<sup>3</sup> The argument is not applicable to losses of applied fertilizers. Granular fertilizers are not inert.

<sup>4</sup> At the Soil and Water Conservation Experiment Station, Bethany, Mo.

The data on 1-year's eroded materials do not give a measure of the degree to which repeated annual data would depart from the textural distributions and organic-matter content that are reported. There is evidence, however, that establishes a reasonable validity for the available data in the use to which they are put. The fact that the samples of eroded material represent from 16 to 35 individual runoffs, and that consistent relationships can be shown to exist between the amounts of runoff and the distribution of the fractions, assures the authors that the major differences exhibited among the eroded materials are not accidental. That these differences are due to sorting, and are not inherent from similar fraction patterns in the plot soils, is established by the fact that the soil-fraction differences between plots do not follow through in the eroded material, even though the soil-fraction differences were found to be significant and highly significant in the cases of sand and silt.

TABLE 1.—*Mechanical analyses of plot soils and of the corresponding material eroded during 1 year*

Textural separates (Mm.)	Plot cover and No.				
	Red clover 1	Rye-fallow 2	Alsike 3	Soybeans 4	Fallow 5
	Percent	Percent	Percent	Percent	Percent
2.0-1.0 .....	2.11	2.39	1.96	1.82	2.67
1.0-0.5 .....	3.53	3.40	3.13	3.36	3.56
0.5-0.25 .....	4.32	4.28	3.93	4.58	4.58
0.25-0.10 .....	14.11	13.46	13.85	14.92	14.18
0.10-0.05 .....	14.90	15.34	14.31	14.96	15.65
Sand 2.0-0.05 .....	38.97	38.87	36.51	39.64	40.64
Silt 0.05-0.002 .....	43.01	43.09	45.12	41.46	40.61
Clay 0.002 .....	18.02	18.04	18.37	18.90	18.75
Organic matter .....	2.12	1.89	2.16	2.02	1.70

ANALYSIS OF ERODED MATERIAL					
2.0-1.0 .....	2.58	1.89	4.37	2.68	2.28
1.0-0.5 .....	4.75	2.66	6.35	4.61	3.90
0.5-0.25 .....	5.67	3.01	6.66	6.09	3.90
0.25-0.10 .....	17.10	9.55	16.55	15.69	13.34
0.10-0.05 .....	13.95	11.74	11.65	12.69	11.03
Sand 2.0-0.05 .....	44.05	28.85	45.58	41.79	34.45
Silt 0.05-0.002 .....	41.15	51.52	39.85	38.19	44.36
Clay 0.002 .....	14.80	19.63	14.57	20.02	21.19
Organic matter .....	4.04	2.12	2.92	2.72	1.91

The analyses of the eroded materials show wider divergences than do those of the soils. In comparison with the soil, the organic-matter content of the eroded material is increased in every case, in some cases markedly so. The percentage of sand in eroded material is shown to be as much as 9 percent less or more than the sand content of the corresponding soil. That it should ever be more, on the basis of a year's erosion losses, is somewhat surprising. It is more surprising that the excessive sand content occurs in the soil losses from plots under a cover of red or alsike clover, and that the lowest sand content occurs in the material of the fallow plots. The anomaly is emphasized in table 2, where the percentages of sand in the soil losses are compared to the amounts of soil lost by erosion from the different plots.

In an earlier investigation some data that showed the same trend as that indicated above were discarded by one of the authors on the assumption that sampling had been at fault. In the present case there is no reason to doubt either the efficacy of the eroded-material sampling or the results of the analyses. If the data are accepted, two conclusions must be drawn: (1) The laws governing the transportation of particles by water cannot be used indiscriminately to account for the composition of eroded material; and (2) the increased organic-matter content of eroded material is not due solely to the presence of increased amounts of silt or clay.

TABLE 2.—Comparison of sand content of eroded material and corresponding annual erosion losses

Plot No.	Cover	Sand in soil	Sand in washoff	Soil loss per acre	Rainfall lost as runoff
		Percent	Percent	Pounds	Percent
1.....	Red clover.....	38.97	44.05	215	5.96
2.....	Rye-fallow.....	38.87	28.85	28,343	11.33
3.....	Alsike.....	36.51	45.58	383	5.20
4.....	Soybeans.....	39.64	41.79	192	1.35
5.....	Fallow.....	40.64	34.45	61,358	18.54

An explanation of textural variability is presented that will be supported later by data on individual soil losses. Under low rainfall and on a permeable soil, conditions are favorable for the total absorption of all precipitation. If, under these conditions, there is any selective movement of fine material, such movement must be downward, mainly through channels and fissures, rather than across the surface of the soil (1, 4). An increase in precipitation to the point where runoff begins does not preclude a continuation of a downward movement of the fines, or a tendency to develop a superficial sandy layer at the soil surface. Consequently, conditions may arise, as has been the case, apparently, on plots 1, 3, and 4, where eroded material actually may be coarser in composition than the eroding soil, as a result of the downward movement of the fines and the transverse movement under subsequent rainfall of the coarser material.

On the plots under investigation where the above-described behavior has been in evidence, erosion losses and the percentages of rainfall lost as runoff have been inconsequential. On the fallow plots where higher percentages of water have been lost in runoff, erosion has followed the pattern ordinarily expected. That is to say, the larger amount of erosion has resulted in material of coarser texture, but in neither case does the composition of the eroded material appear to be as coarse as the material from which it is derived. When the relative quantities of eroded soil from the different plots are considered it appears that under local conditions the general tendency has been to remove the fines selectively.

Complete mechanical analyses of the soil losses resulting from the separate rains have not been made. However, where sufficient amounts of sample were available, a single wet-sieve separation was made at 200 mesh for the separate losses from plot 5. The results of these sieve analyses, together with the data on rainfall and erosion losses are given in table 3.



TABLE 3.—Analyses of materials eroded from plot 5, and related erosion data

Dates	Precipitation		Intensity per hour			Runoff		Soil loss		Mechanical separates		Organic matter of separates	
	Amount Inches	Duration Hrs: Min.	Average Inches	Maximum in—			Depth Inches	Precipitation Percent	Per acre Pounds	Per cubic foot of water Pounds	Organic matter Percent	Coarser than 200 mesh Percent	Finer than 200 mesh Percent
				5 minutes Inches	15 minutes Inches	30 minutes Inches							
Jan. 25.....	0.67	6 : 00	0.11	0.42	0.22	0.20	1.1608	1240.00	1,679.9	0.288	3.41	2.36	3.44
Feb. 9.....	.50	10 : 10	.05	.72	.44	.28	.084	16.80	200.7	.656	3.86	5.38	3.65
Feb. 13.....	.79	11 : 20	.07	.36	.16	.16	.169	21.39	308.0	.503	4.21	8.45	3.18
Mar. 23.....	.32	32 : 40	.48	2.64	.96	.60	.020	6.25	208.2	2.892	2.60	3.13	3.13
Mar. 31.....	.36	25 : 15	.86	2.04	1.36	.60	.164	45.56	4,821.6	8.093	2.68	1.67	2.68
Apr. 13.....	.18	17 : 00	.72	1.68	1.72	.38	.108	60.00	2,073.3	5.204	2.89	1.65	2.44
Apr. 22.....	.72	5 : 10	.14	.60	.46	.12	.003	13	18.4	1.518	1.88	1.09	1.06
May 15.....	.74	17 : 00	.05	.18	.12	.12	.001	3.78	20.6	4.790	1.83	.69	2.81
June 7.....	.22	5 : 55	.25	3.60	1.78	1.06	.028	10.45	212.1	2.072	2.06	.98	2.60
June 11.....	.48	5 : 20	.00	.84	.52	.22	.070	14.58	624.7	7.389	2.29	1.10	2.41
June 17.....	.20	45 : 08	.27	.84	.36	.52	.002	4.948	515.6	2.917	2.68	1.11	2.89
June 18.....	.14	1 : 30	.08	1.05	.72	.00	.044	31.43	793.5	4.048	2.35	1.18	2.46
July 14.....	.15	1 : 10	.28	2.16	.90	.00	.005	3.33	63.3	10.153	1.73	1.00	2.70
July 23.....	.34	35 : 35	.59	2.64	1.04	.66	.028	8.21	79.3	3.488	2.27	1.20	2.28
July 25.....	.14	30 : 30	.28	.84	.36	.28	.003	1.43	351.7	31.40	2.36	.95	2.53
July 28.....	.28	12 : 00	.37	3.00	1.00	.56	.021	7.50	40.2	5.398	2.10	1.05	2.73
Aug. 10.....	4.46	1 : 35	.26	6.00	3.84	2.06	2.400	53.81	47,804.3	3.494	1.70	.66	2.07
Aug. 30.....	.41	4 : 10	.84	1.96	1.36	.78	.003	1.46	99.5	4.013	2.01	.97	2.31
Sept. 12.....	.33	4 : 10	.08	.84	.48	.28	.003	.91	12.4	1.203	2.85	1.40	3.05

1 Runoff greater than the recorded precipitation was caused by melting snow.

Inspection of these data show that under the small rains characteristic of this location, there is no apparent correlation for the texture of the eroded material with the intensity and duration of rainfall, the soil-water ratio of the runoff, or the amounts of the erosion losses of either soil or water. Elimination of these factors makes it appear that surface conditions, modified by cultivation, and the residues, or the downward movement of colloid that results from prior rain, have been most effective in governing the composition of the eroded material.

Attention should be directed specifically to the data for the storm of August 10. This one rain was outstanding, both with regard to intensity and total precipitation. It accounts for 78 percent of the annual erosion loss. Here the total losses were sufficiently great to discount any minor surface variations. As a result, the texture of the eroded material approximates very closely the texture of the plot soil.<sup>5</sup>

One trend in the data that appears to be fairly definite is that coarser textured material was eroded during summer rains. All the storms that produced eroded material containing more than 35 percent of material coarser than 200 mesh occurred under conditions favorable to permeability between April 20 and September 30, and caused small erosion losses. This behavior is in harmony with the results of the annual erosion on plots 1, 3, and 4 (table 2), where a tendency to produce sandy eroded material was indicated.

Wherever the eroded material contains less than 20 percent of material coarser than 200 mesh, it has been caused by fall or winter rains. One of these (January 25) produced a relatively large amount of erosion. Two other storms that produced a relatively large erosion of fines occurred March 31 and April 13. All three of these storms occurred under like circumstances in that the ground was wet at the time of their occurrence and previous freezing and thawing had tended to destroy the aggregates at the soil surface. Conditions therefore were favorable to the erosion of textural separates. Any tendency of fines to move downward into the soil was at a minimum, and the percentage of rainfall lost by runoff was correspondingly high.

#### SOURCES OF ORGANIC MATTER IN ERODED MATERIAL

Because the organic matter of soils is known to be associated mainly with the finer fractions, it is not unreasonable to assume that when the organic-matter content of the eroded material is high as compared to that of the eroding soil, the increase in organic matter is due to an increase of fines in the material eroded. This assumption has been strengthened by the fact that high organic matter usually accompanied small runoffs. In data already presented (table 1) evidence is given to show that the assumption is fallacious, that organic-matter content need not reflect textural composition, although obviously textural composition is a factor in determining the amount of organic matter present.

To assume that dispersed soil colloidal material abnormally rich in organic matter has been removed selectively by the erosion process in those cases in which organic-matter content does not result directly from textural composition is contrary to technical experience (2). Hide and Metzger (5) have shown that relatively high organic matter

<sup>5</sup> See table 4 for comparable soil data.

is associated with the better aggregated soil fractions. If effective differential erosion takes place on an aggregation basis, eroded material should contain less rather than more organic matter than the parent soil. In view of the evidence, the most logical assumption seems to be that the excess organic matter in eroded material, insofar as it is not a result of texture, is due to organic debris removed by erosion.

The data of table 4 support this conclusion to the extent that the annual eroded material coarser than 200 mesh is richer than the corresponding soil fraction in organic-matter content. In these fractions it was evident visually that most, if not all, of the organic matter present consisted of discrete fragments of animal or vegetable origin. The only instance where soil and eroded material contained reasonably similar amounts of such material was on the fallow plot 5, where lack of vegetal cover and highest erosion losses tended to reduce debris effects.

A separation at 200 mesh does not prevent the inclusion of debris material in the finer fraction; consequently it is not surprising to find some rather wide differences between soil and eroded material in the organic-matter content of these fractions. Some of this difference probably is due to varying proportions of very fine sand, silt, and clay, a point that could not be checked in the present investigation. That the differences are due in part to the inclusion of debris is indicated by the fact that the order of organic-matter content in the eroded material is the same in both the fine and the coarse fractions. Moreover, on the fallow plots, 2 and 5, where greatest erosion occurred, the organic-matter content of the fine fraction of the eroded materials approaches the limits set by the analyses of the fine soil fractions.

TABLE 4.—Organic-matter content of coarse and fine fractions of the plot soils and of the corresponding material eroded during the year

ANALYSIS OF SOILS					
Plot No.	Organic matter	Mechanical separates		Organic matter of separates	
		Coarser than 200 mesh	Finer than 200 mesh	Coarser than 200 mesh	Finer than 200 mesh
	Percent	Percent	Percent	Percent	Percent
1.....	2.12	30.5	69.5	0.98	2.44
2.....	1.89	30.0	70.0	.43	2.40
3.....	2.16	28.0	72.0	.97	2.77
4.....	2.02	30.5	69.5	.61	2.68
5.....	1.70	30.1	69.9	.56	1.95

ANALYSIS OF ERODED MATERIAL					
1.....	4.04	41.5	58.5	2.74	4.71
2.....	2.12	22.0	78.0	1.26	2.31
3.....	2.92	42.0	58.0	1.89	3.47
4.....	2.72	33.0	67.0	1.45	3.22
5.....	1.91	30.1	69.9	.79	2.24

Enough samples were available from plots 2 and 5 to make separate analyses of the 200-mesh material from the rain of August 10. The erosion occasioned by this rain was sufficient to mask debris effects. The material from plot 2 ran 2.27 percent and the material from plot 5 ran 2.07 percent in organic-matter content.

In table 3, data from the type of analysis just discussed have been reported for the sieve fractions from the plot 5 individual soil losses. Here, where effects of cover are absent, there is an evident relationship between organic-matter content and the proportion of the textural separates present in the eroded material. But there is evidence of debris effects also in the high organic-matter content of some of the coarser fractions. The source of this may be wind-borne material. This plot had been kept completely fallow for 3 years prior to the time of this investigation.

It has been emphasized that a constancy of colloid composition may be assumed in dealing with the eroded-material fractions. To test the point and show that a high organic content in the fines of this material is in no way associated with the segregation in the eroded material of inorganic colloidal material especially rich in organic matter, some colloidal material was extracted both from the soil and from a series of soil losses. Duplicate extraction and analyses of soil colloid gave organic-matter content of 3.17 and 3.32 percent; colloid from separate soil losses ran 3.14, 3.29, 3.34, and 2.96 percent.

#### DISCUSSION

The results of the present study, together with those of former investigations, furnish a basis for evaluating the variability of eroded materials. Evidence that is available indicates that under severe erosion the eroded materials tend to approximate the composition of the eroding soil, that it is in effect "removed layer by layer." With more moderate runoffs there is a selective removal of the finer fractions. Small local depositions of sand on the soil surface may be swept off by later rains, but if frequent cultivation constantly presents a fresh surface to the sorting action of running water, a continued removal of fines may be expected.

On relatively permeable soils, where infiltration represents a high proportion of the total precipitation, the downward movement of fines apparently results in a superficially sandy surface layer. The removal of this material by runoffs of slightly greater intensity results in small erosion losses of relatively coarse texture.

The widest range of composition between soil and eroded material is to be expected from loams or sandy soils. The removal of "finer fractions" by erosion from a soil composed entirely of silt and clay seems highly improbable. Moreover, the most effective sorting by comparable runoffs may be expected to take place on weakly aggregated or single-grained soils, since the removal of textural separates implies a break-down of the texturally heterogeneous soil aggregates.

That soils generally are not markedly affected texturally by the variability of eroded materials is evident in part from the fact that the heaviest erosion losses show the least variability. A simple example will illustrate the effect of smaller losses. An erosion loss of 10 tons per acre that contains 10 percent less sand than the original soil results in an accumulation of sand of but 1 ton per acre, or enough to increase the sand content of the surface soil by about one-tenth of 1 percent.

The silt and clay content obviously drop by the same amount, but changes of this order are negligible, except on excessively sandy soils or for their cumulative effect. The data of Scarseth and Chandler (9)



illustrate the point. These investigators found that under the conditions of their experiment, a selective erosion of fines took place that amounted to only 3 percent of the whole soil, but nevertheless was 33.3 percent of the clay fraction. To infer, however, that continued removal of excess fines by erosion must inevitably cause a surface soil to become lighter in texture is fallacious. That can happen only if the subsoil that replaces erosion losses is lighter in texture than the eroded material.

Because of this circumstance the assumption should not be made that erosion losses always are relatively more damaging if they contain high percentages of fine materials. The assumption is based on the fact that the available plant nutrients of the soil are confined mainly to the finer fractions. It overlooks entirely the equally well-known fact that soil structure and tilth, which are dependent in part on texture, also are important in maintaining productivity. Podsolc soils generally tend to become heavier as the result of erosion. On soils of this type, particularly on those that have a low organic content, experience may show that a selective removal of fines is less damaging than the removal of the same amount of sandy material.

The point determined in the present investigation, that small erosion losses may be sandy in character, may not be of more than theoretical interest because of the small amounts of erosion that are involved. It seems to bear, however, on the data presented by Hester and Shelton (4), which show a downward movement of silt and clay from the surface horizon of a soil that amounted to 79 tons per acre, as the result of long-time cropping and fertilizer practices. This result contrasts so markedly with the results obtained by Scarseth and Chandler that it seems necessary to postulate essentially different types of erosion for the two experiments.

The losses of organic matter caused by erosion are variable both in amount and character. They tend to be high in proportion to the total amount of soil and the proportion of fines that are lost. It is to be presumed, however, that the loss of well-decomposed and relatively stable humus intimately associated with the organic-inorganic soil complex, closely follows the textural composition of the eroded material. Losses of organic matter in excess of this amount may vary in character from bacterial gels and the mycelia of fungi to coarse and fibrous residues of vegetation. Small runoffs contain higher proportionate amounts of such materials.

These assorted organic materials may be characterized as the more active fraction of the eroded organic matter. The variable results of such losses on the remaining soil may be judged by the effects of applications of similar amounts of similar materials, litters, manures, or composts.

It is well known that organic materials applied to the soil tend to disappear in a relatively short time as a result of bacterial decomposition and oxidation. The organic debris that is carried away by erosion undoubtedly is destined for the same fate, and its presence therefore tends to exaggerate the permanently characteristic differences that distinguish a soil from its eroded material. The removal of active organic matter of high fertility value may have an immediately deleterious effect on the remaining soil of somewhat temporary character.

Losses of relatively stable humus are cumulative in effect, and therefore are of greater importance. Soil humus is not replaced easily. Its capacity to release food and energy for plant growth and bacterial activity is small as compared with that of green manures or freshly applied composts; nevertheless it maintains an ability to serve as a storehouse of plant food by reason of its amphoteric character and high base-exchange capacity, and so regulates the supply of nutrients available for plant growth. Equally important, it affects the physical character and tilth of the soil, and tends to buffer and extend the zone of conditions favorable to maximum plant growth. The variability of eroded material that supports an excessive loss of fines becomes relatively more damaging in proportion to the rate at which it lowers the humus content of the remaining soil.

#### SUMMARY

A series of soil losses and the corresponding plot soils have been analyzed texturally and for their organic-matter content.

Since rainfall was light, soil and surface conditions, rather than intensity and duration of rainfall, appeared to govern the textural composition of the eroded material. Under conditions favorable to infiltration and downward movement of fines, eroded material has been produced that is coarser in texture than the corresponding soil; under conditions of greater impermeability, eroded material has been produced that is finer in texture than the corresponding soil.

These differences tend to disappear as erosion increases, and the composition of the eroded material approaches the composition of the soil.

Argument has been advanced to show that a disproportionate removal of fines need not be more serious than the removal of the same amount of soil in its total.

Irrespective of texture, eroded material has been shown to be somewhat higher in organic-matter content than the eroding soil, especially where small runoffs are incurred. This anomaly has been shown to be the result of organic debris that is removed preferentially by the eroding process.

If the effect of organic debris is discounted, colloidal material removed by erosion has essentially the same organic-matter content as the colloidal material of the eroding soil.

The textural separates of a soil are essentially identical with comparable separates from its eroded material and, except for deviations due to organic debris or temporary physical conditions, all properties in which eroded material may differ from the soil must be the direct result of the relative proportions of separates that are included in the material.

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211  
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Vol. 65

4/10/42  
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## CONTENTS

	Page
Influence of Controlled Environment and Nutrition on the Resistance of Garden Pea to Fusarium Wilt (Key No. Wis-133) - - - - -	221
WILBUR T. SCHROEDER AND J. C. WALKER	
Influence of Heredity and Other Factors on 180-Day Weight in Poland China Swine (Key No. Iowa-32) - - - - -	249
J. A. WHATLEY, Jr.	



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## INFLUENCE OF CONTROLLED ENVIRONMENT AND NUTRITION ON THE RESISTANCE OF GARDEN PEA TO FUSARIUM WILT<sup>1</sup>

By WILBUR T. SCHROEDER, formerly fellow, and J. C. WALKER, professor of plant pathology, Wisconsin Agricultural Experiment Station

### INTRODUCTION

Resistance to fusarium wilt of pea (*Pisum sativum* L.), first noted by Linford (7),<sup>2</sup> was demonstrated by Wade (19) to behave as a single dominant Mendelian character. Although the disease has declined in importance with the development of resistant varieties, it remains of fundamental biologic interest in that it is in some respects an extreme case of the specialized parasitism common in the vascular fusarioses and differs noticeably in its temperature relations from other diseases in this group. Linford (7) and Snyder (16) showed that the growth of the pathogen, *fusarium oxysporum* f. *пси* (Lindf.) race 1 S. & H. (*Fusarium orthoceras* App. and Wr. var. *пси* Linford), on various media is similar to that of other vascular fusaria. Linford (7), however, found the optimum soil temperature for development of pea wilt to be distinctly below that for the growth of the fungus on potato-dextrose agar, while that in other vascular fusarioses is close to the optimum for linear growth of the parasite on agar substrate.

Walker (20) demonstrated that host resistance to cabbage yellows (*Fusarium oxysporum* Schlecht. f. *conglutinans* (Wr.) S. & H.), like that to pea wilt, is controlled by a single dominant Mendelian gene. Cabbage yellows, however, differs from the latter disease in that homozygous resistant individuals develop certain atypical symptoms at soil temperatures of 26° to 30° C. (1, 22). At the same temperature range Linford (7) claimed that pea wilt was much reduced in severity. Anderson and Walker (1) found that penetration of the resistant cabbage occurred through the epidermis and cortex of the root and hypocotyl, but that the organism seldom reached the stele. Walker and Snyder (23) observed that, in contrast to *F. oxysporum* f. *conglutinans*, the pea-wilt organism becomes established with difficulty in certain soil types.

The purpose of the present investigation was to study the effect of certain environal and nutritional factors on the development of pea wilt, particularly where the soil factors were eliminated. This was accomplished by growing a susceptible and a resistant variety of the host in artificially infested sand in which temperature and nutrition

<sup>1</sup> Received for publication January 14, 1942. This investigation is part of a study of the nature of disease resistance in plants, supported in part by the Wisconsin Alumni Research Foundation. Some assistance was provided from the personnel of Federal Work Projects Administration Project No. 65-1-53-2349.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 246.

could be regulated. Under these conditions a better understanding of the host-parasite relationship could be acquired which might in turn throw more light on the nature of disease resistance. All experiments were conducted in the greenhouse or laboratory at Madison, Wis.

#### MATERIALS AND METHODS

Two varieties of pea, Davis Perfection (wilt-susceptible) and Wisconsin Perfection (wilt-resistant), were used throughout. The isolate of *Fusarium oxysporum* f. *pisi* race 1 was obtained from W. C. Snyder of the University of California and all inocula used in this study were obtained from repeated subtransfers of a single microconidial line derived from that culture. Five-pound glazed crocks, 5 inches high, were used. To each crock was added approximately 1,200 ml. of dry, fine, white silica sand, which previously had been washed in several

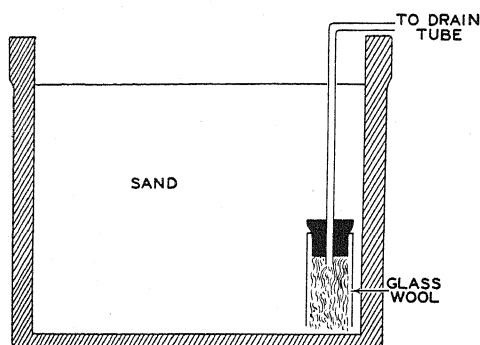


FIGURE 1.—Siphon drain for sand-culture crocks. The 2-inch piece of glass tubing filled with glass wool served to prevent the sand from clogging the drain.

changes of hot water followed by several rinsings in distilled water. Each crock was supplied with a siphon through which the sand was drained (fig. 1). The sand contained in each crock was wetted and sterilized for 6 to 8 hours at 15 to 18 pounds' pressure on 2 alternate days, and just prior to planting it was washed twice with distilled water by flooding each crock and draining through the siphon.

Modifications of the nutrient solution formulated by Hoagland and Snyder (4) were used throughout. In the temperature experiments the basal solution was diluted to one-tenth concentration. The studies on the effect of nutrient concentration involved the dilute solution, the basal solution, and multiples of the latter. Hereafter, the diluted solution is designated as 0.1H, the basal as 1H, three times the basal as 3H, and five times the basal as 5H. No attempt was made to control the pH of the solutions. On alternate days approximately 500–600 ml. of nutrient solution was added to each crock and the excess solution drained off immediately.

The composition and molarity of the nutrient solutions at the basal concentration used in the sand-culture experiments were as follows:

Composition and molarity	Milliliters to make 10 liters of nutrient
$\text{Ca}(\text{NO}_3)_2$ , 1M	50.0
$\text{KNO}_3$ , 1M	50.0
$\text{MgSO}_4$ , 1M	20.0
$\text{KH}_2\text{PO}_4$ , 1M	10.0
$\text{NaCl}$ , 1M	5.5
A-Z <sup>1</sup>	2.0

<sup>1</sup> The A-Z stock solution was composed of the following made up to 1,000 ml. with distilled water:  $\text{H}_3\text{BO}_3$ , 2.818 gm.;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.40 gm.;  $\text{ZnCl}_2$ , 0.030 gm.;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.390 gm.; and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 5.000 gm.



Inoculum was obtained by growing the fungus in 1-liter Erlenmeyer flask containing 200 ml. of Czapek's solution modified according to the following formula:  $\text{KNO}_3$ , 3gm.;  $\text{KH}_2\text{PO}_4$ , 1.0 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 gm.;  $\text{KCl}$ , 0.5 gm.;  $\text{FeSO}_4$ , trace; dextrose, 30 gm.; water, 1,000 ml. The inoculated flasks stood for 48 hours with frequent shakings each day, after which they were placed in a mechanical shaker for 3 to 4 days. After this period of agitation, the contents of each flask consisted of a very heavy suspension of microconidia and hyphal fragments. Inoculation was accomplished by adding 100 ml. of the original mycelial and spore suspension combined with 100 ml. of sterile 2-percent dextrose solution to each crock, draining the excess liquid, and thoroughly mixing the sand in each crock. Each control crock received 200 ml. of the sterile dextrose solution. The peas were then planted, usually eight seeds per crock, and flooded with the nutrient solution, which drained off immediately. The crocks were then placed in the respective temperature tanks. A second inoculation was made 3 to 4 days after emergence by adding 100 ml. of the mycelial and spore suspension to each crock, draining off the excess liquid, and flushing immediately with the nutrient solution.

In order to insulate the sand and thus maintain more complete temperature control, a circular  $\frac{1}{2}$ -inch wire-mesh screen was suspended about  $\frac{1}{2}$  inch above the surface of the sand just after planting. A very thin layer of nonabsorbent cotton was placed over the screen. When the plants emerged through the mesh, the cotton was tucked around them. This method was more satisfactory than the use of granulated cork since the latter became wet when the nutrient solution was added to the sand and thus provided a suitable substrate for air-borne contaminants, particularly *Cephalothecium roseum* Cda.

#### SYMPTOMS AND DISEASE CRITERIA

The symptoms of pea wilt have been described in detail by Linford (7, 8, 9, 10). The initial and most characteristic sign of this disease in susceptible plants is a downward curving of the stipules and leaflets, accompanied by a slight yellowing of the leaves and a superficial grayness suggesting an excessive development of waxy bloom. The lower internodes increase in diameter and the entire stem becomes rigid. After this stage the plant may wilt abruptly at the top, and the stem shrivel downward or, particularly under low soil-temperature conditions, it may turn yellow and wither slowly, leaf by leaf. Linford (9) has described, in plants grown aseptically in tubes of soil inoculated with a pure culture of the fungus, distortion and wilting of the leaflets followed by sudden collapse of the plant, sometimes accompanied by a water soaking of the collapsed parts and extensive cortical decay of the roots.

The progress of the disease was noted daily. Data were taken on the appearance of initial symptoms, complete wilting, and death. Complete wilting was designated as that stage in which all stipules and leaflets except those enclosing the terminal bud were either definitely wilted or dead. Indices were calculated according to the method used by Virgin and Walker (17, 18) in which the index represents the average number of days from sowing to the particular stage of the disease concerned. The index for the initial appearance of symptoms was designated as the initial-disease index; that for

complete wilting, the wilt index; and that for death, the death index. On this basis, the more rapid the disease development became, the lower the index. Since neither complete wilting nor death occurred in all resistant plants, a disease-development index was used as the disease criterion. At a given period, plants were removed from the sand and divided into five arbitrary classes designated as follows: 0, plants healthy; 1, no apparent stunting, but an incurving of stipules and leaflets, accompanied by off-color; 2, moderate stunting, incurving of stipules and leaflets and off color apparent to within three or four nodes of terminal bud; 3, severe stunting, only one to three uppermost nodes with green leaves remaining and possessing the characteristic incurving and off color (such plants approached the condition of complete wilting); 4, complete wilting and death. The disease-development index was calculated on the basis of the above classes by using the class figures as weighted values.<sup>3</sup>

#### ENVIRONMENTAL STUDIES

Linford (7) indicated that the most rapid and severe development of pea wilt occurred at a soil temperature of 21° to 22° C. He based his conclusions on the percentage of diseased plants at the various soil temperatures on successive-day intervals following planting. Using a wilt index, Virgin and Walker (17) compared the soil-temperature relations of the near wilt disease of pea (*Fusarium oxysporum* Schlecht. f. *pisi* (Sny.) race 2 S. and H.) with those of pea wilt and corroborated Linford's observations on wilt, although the differences between 20°, 24°, and 30° were not very large.

Early experiments involving a study of methods for obtaining uniform infection of peas in sand artificially infested with the pea wilt organism indicated that the optimum temperature for disease development was not convergent with that in soil. Furthermore, it was observed that at temperatures of 24° C. and higher, resistant plants were stunted, and they developed symptoms characteristic of the initial stages of the disease described by Linford (7) for susceptible plants. Two experiments were therefore set up to determine the influence of sand temperature on development of the disease in susceptible and resistant varieties. The first experiment was started in the greenhouse on December 2 and final notes were taken on January 17; the second experiment was started on January 31 and concluded on March 18. The chief variable in the two experiments, therefore, was light, the length of day being shorter and the intensity of light usually lower in the first experiment. In experiment 1 a parallel series was run in virgin soil in which inoculum increased on sterilized barley kernels was incorporated at approximately the same rate as that used by Snyder (16). The soil was maintained at approximately 60 percent of its water-holding capacity by the addition of distilled water on alternate days. Uninoculated soil served as a control. Three inoculated and two control crocks of each variety in each substrate were planted. Crocks and tanks were arranged

<sup>3</sup> To obtain the index the number of plants in each class was multiplied by the class number and the sum of the products of each class was then multiplied by 100. The product was then divided by 4 times the total number of plants in each treatment to obtain the disease-development index. Thus, when all the plants were healthy, the index was 0, when all the plants were completely wilted the index was 100, while intermediate stages of the disease were represented by intermediate index figures. It is important to note that the disease-development index figure increases in value with increase in disease severity while in the initial-disease index, wilt index, and death index the reverse relation holds.

at random to compensate for any variation in quantity and intensity of light in the greenhouse. Tank temperatures were maintained at 15°, 18°, 21°, 24°, 27°, and 30°, and the cotton insulation in each crock permitted these temperatures to be held to within ½° in the sand and soil. The air temperature was held between 22° and 24°, except for about 4 hours during midday on sunny days, when it went as high as 28°.

#### INFLUENCE OF TEMPERATURE ON THE DISEASE IN SUSCEPTIBLE PLANTS

The influence of sand temperature on the development of wilt was markedly different from that observed by Linford (7) for soil. From the data given in table 1 it is to be seen that the time required for complete wilting to occur was significantly less as the temperature rose from 15° to 30° C. The difference between the wilt indices at 27° and 30° was not significant in experiment 1 and just barely so in experiment 2. The time required for complete wilting in the latter was the longer at each temperature. Thus the optimum temperatures for complete wilting in these trials were 27° and 30°.

TABLE 1.—Wilt indices of Davis Perfection (susceptible) peas in two different experiments at various sand temperatures

Experiment No.	Duration of experiment	Wilt index (days) at sand temperatures of—						Minimum significant difference (19:1) <sup>1</sup>
		15° C.	18° C.	21° C.	24° C.	27° C.	30° C.	
1.....	Dec. 2 to Jan. 12.....	31.3	27.9	24.2	20.6	16.8	16.5	2.10
2.....	Jan. 31 to Mar. 18.....	41.1	33.6	29.8	25.6	19.4	18.3	1.03

<sup>1</sup> Minimum difference required for significance between the indices at various temperatures in a given experiment.

The progress of disease development (fig. 2) showed the same trend in the three indices, although the intervals between the three stages of wilt development decreased slightly with the rise in temperature.

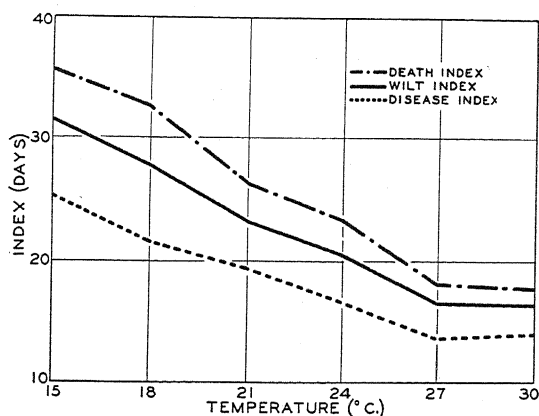


FIGURE 2.—Comparison of the initial-disease index, the wilt index, and the death index in Davis Perfection (susceptible) peas at various temperatures in artificially inoculated sand.

While any one of the three stages could be used as a disease criterion, complete wilting appeared to be the one most accurately determined. A comparison of the initial-disease indices in sand and soil presents an interesting picture (fig. 3). As previously indicated, the differences among the indices at the various sand temperatures were all significant except at 27° and 30° C. In the soil, however, the appearance of initial symptoms at 18°

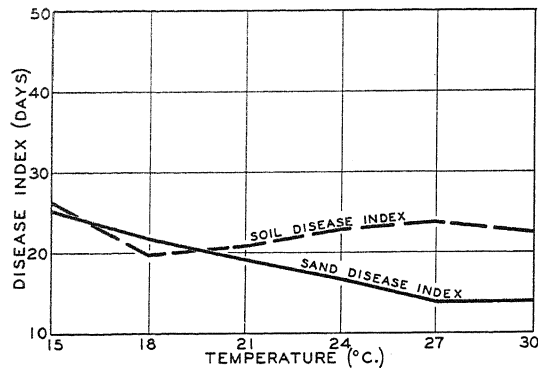


FIGURE 3.—The influence of temperature on the initial-disease index in Davis Perfection (susceptible) peas grown in artificially inoculated sand and soil at various temperatures.

sult that the optimum temperature for disease development in sand was much higher than that in soil.

#### INFLUENCE OF TEMPERATURE ON THE DISEASE IN RESISTANT PLANTS

Several attempts have been made to induce the development of wilt in resistant varieties. Linford (9) failed to break down inherent resistance by pruning and otherwise injuring the roots of plants already established in wilt-infested soil. Decortication of the roots of transplants before placing in infested soil, as performed by Linford (9) and Wade (19), resulted in no manifestation of the disease other than a dropping of a few of the lower leaflets, although both workers state that the fungus was found sparingly in the cortex but not in the stele of the root. A similar situation was observed by Walker (21) in the resistant Alaska variety grown in soil and on soil-extract agar. Snyder (16) observed leaf symptoms on the resistant Bruce variety suggestive of wilt, but failed to recover the fungus from such plants. By applying a barley-grain culture of the fungus to wounds in the aerial parts of resistant and susceptible varieties, Linford (10) obtained symptoms which simulated those typical of the disease in 6.7 and 13.3 percent of the plants, respectively. The fungus, however, extended only a few millimeters vertically from the wound. In the two experiments previously described Wisconsin Perfection (resistant) peas were grown in a parallel series. Disease development in this variety will be considered next.

The symptoms in resistant plants were found to differ from those in susceptible plants in degree rather than in kind. The initial symptoms were quite similar in that the normal color of the lower leaflets and stipules gave way to a slight yellowing, followed by withering and death. As a given leaflet withered younger stipules and leaflets assumed a superficial grayness and became incurved about three nodes in advance of that at which leaves were dying. The dead leaves abscised at about the same rate as those of susceptible plants grown at 15° and 18° C. but more readily than those of susceptible plants grown under temperatures conducive to rapid wilting. Under ideal conditions, these symptoms gradually proceeded to the

and 21° was significantly earlier than at any of the other temperatures. At 15° the symptoms were decidedly retarded, while at 24°, 27°, and 30° they were intermediate in rate of development between those at 15° and at 18° and 21°. Thus at 15°, 18°, and 21° the disease developed at approximately the same rate in both sand and soil, but at 24°, 27°, and 30° a divergence occurred with the re-



top of the plant until all fully expanded leaves had withered and died. As in susceptible plants, the lower internodes increased in diameter and the entire shoot became more rigid, while stunting and shortening of the internodes were also quite characteristic. Under less favorable conditions, such as lower temperatures, the characteristic off color and incurving of the stipules and leaflets developed occasionally, but they were confined to the lower half of the stem and occurred without any apparent stunting of the plant. A certain amount of cortical decay existed in the roots of such plants.

The influence of temperature on disease development in Wisconsin Perfection was not as marked nor as uniform as in susceptible Davis Perfection. The initial-disease indices are given in table 2 while the percentages of completely wilted plants at various intervals for each temperature are represented in figure 4. The temperatures of 24°, 27°, and 30° C. were uniformly effective in bringing about early appearance of initial symptoms, while 27° and 30° appeared to be slightly more favorable than 24° for the early development of the complete-wilt stage. The symptoms appeared later at 21° and were greatly retarded at 15° and 18°.

TABLE 2.—The influence of temperature on the appearance of initial symptoms in Davis Perfection (susceptible) and Wisconsin Perfection (resistant) peas grown in artificially inoculated sand

Experiment No.	Duration of experiment	Variety	Initial-disease index <sup>1</sup> (days) at sand temperatures of—					
			15° C.	18° C.	21° C.	24° C.	27° C.	30° C.
1.....	Dec. 2 to Jan. 12..	(Davis Perfection.....	25.3	21.8	19.4	16.8	13.7	14.0
		(Wisconsin Perfection....	31.9	30.2	23.4	14.9	14.4	14.8
2.....	Jan. 31 to Mar. 18..	(Davis Perfection.....	28.2	21.9	18.2	16.2	14.5	14.4
		(Wisconsin Perfection....	33.5	30.5	26.8	20.3	19.3	20.9

<sup>1</sup> Based on the average of 3 replicates.

At the conclusion of experiment 1, those plants that had not wilted completely were removed, washed free of sand, surface-sterilized for 5 to 8 minutes in a 0.9-percent solution of sodium hypochlorite, and nodal platings were made on acidified potato-dextrose agar. A fungus resembling *F. oxysporum* f. *pisi* race 1 with respect to cultural characters was isolated from every plant, although the nodal location of the fungus was irregular. The majority of isolations were from the cotyledonary and second nodes. In some plants the fungus was recovered from as high as the fifth node, but was rarely recovered from that section of the taproot located about 1 cm. below the cotyledonary node.

Forty-five days after planting, those plants of the resistant variety that had not wilted completely in experiment 2 were removed from the sand and the disease-development index was determined prior to planting the nodes on acidified potato-dextrose agar. The indices were as follows:

Temperature (°C.):	Disease-development index
15.....	26.3
18.....	30.7
21.....	47.4
24.....	73.7
27.....	91.3
30.....	92.1

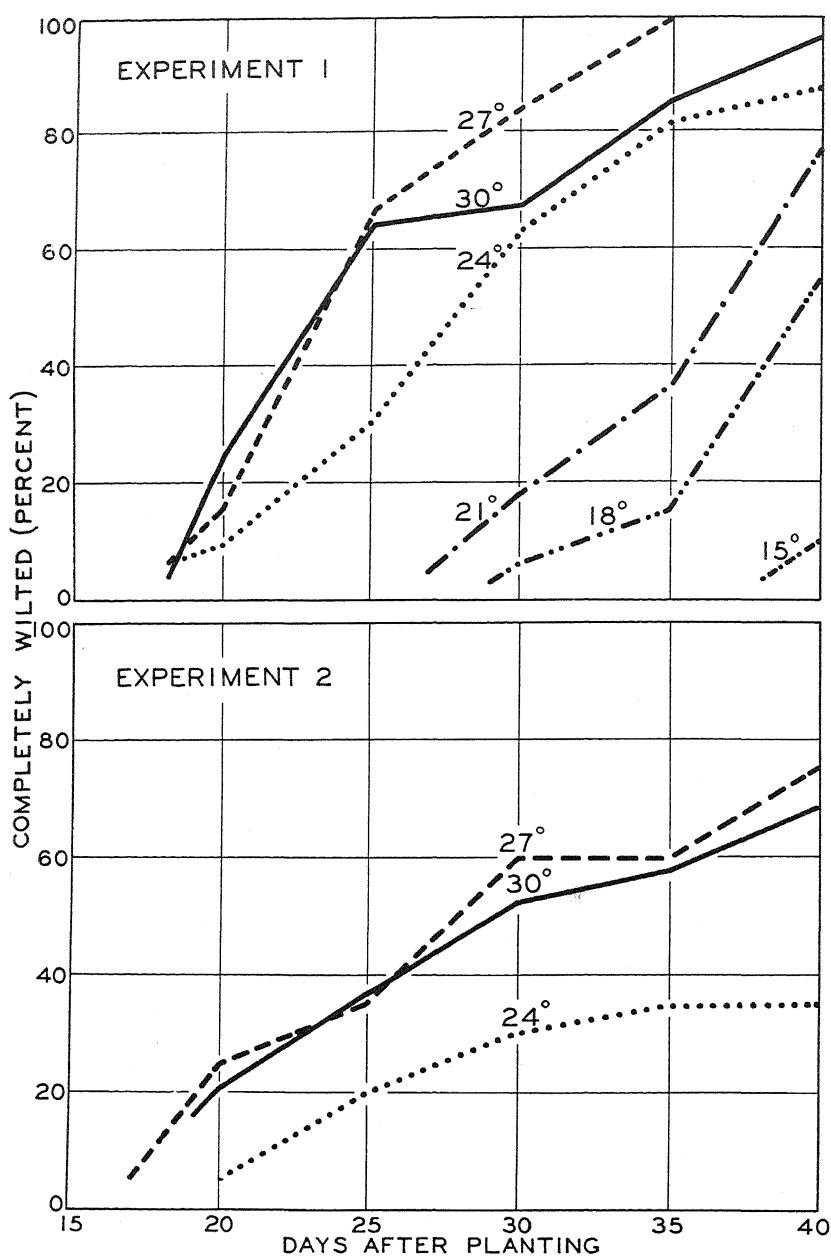


FIGURE 4.—The influence of sand temperature on the percentage of plants reaching the complete-wilt stage in Wisconsin Perfection (resistant) peas grown in artificially inoculated sand.

The most severe development of the disease occurred at 27° and 30° C., with a decrease at 24° and a still further decrease at 21°, while at 15° and 18° the majority of the plants showed merely the characteristic incurving and off color at the lower nodes. The results of the nodal isolations conformed closely to the disease-development indices. Those plants classified as healthy did not yield the organism. In diseased plants at 15°, 18°, and 21° the pathogen had not progressed farther than the second node and was sparse in the root. At 24°, 27°, and 30° the organism was recovered from the roots and second node of all plants and from the fourth, fifth, or sixth nodes of some.

#### INFLUENCE OF AIR TEMPERATURE ON DISEASE DEVELOPMENT

In view of the marked differences effected by various sand temperatures on the development of pea wilt, a study was made of the influence of different air temperatures on the progress of the disease in the susceptible and resistant varieties grown at two constant sand temperatures. Triplicate plantings of each variety were made at sand temperatures of 20° and 28° C. in each of the following air temperatures: 16°, 20°, 24°, and 28°. Control crocks were planted in duplicate. Cotton insulation was used in all crocks. The results in table 3 show no significant difference in wilt indices at the four air temperatures when the sand temperature was 28°. When the sand was at 20° complete wilting was definitely retarded at 16° air, as compared with air temperatures of 20°, 24°, and 28°. Of the last three air temperatures wilt was significantly the more rapid at 24°. However, at air temperatures of 20°, 24°, and 28° the modification of disease expression occasioned by shifting the sand temperature from 20° to 28° or vice versa was greater than that between 20° and 28° air temperature at either soil temperature. The fact that the rate of wilt development in the sand held at 28° was not influenced by air temperature is explained in part by the observation cited elsewhere in this paper that complete wilting in susceptible plants in sand at 27° and 30° was the result of a different set of host-parasite relations than that in plants grown in sand at 24° and lower temperatures.

TABLE 3.—Development of the complete-wilt stage in *Davis Perfection* (susceptible) peas grown in artificially infested sand at two sand temperatures in each of four air temperatures

Sand temperature (° C.)	Wilt index at air temperature of—				Minimum significant difference (19:1)
	16° C.	20° C.	24° C.	28° C.	
20.....	<sup>1</sup> 30.7	27.7	25.9	26.7	1.6
28.....	17.7	18.0	17.8	20.5	(2)

<sup>1</sup> Average of 3 replicates.

<sup>2</sup> Calculated *F* value indicated no significant differences among the four air temperatures.

#### TEMPERATURE IN RELATION TO THE GROWTH OF THE FUNGUS

The optimum temperature for growth of *F. oxysporum* f. *pisi* race 1 as measured by radial expansion on agar media of diverse composition has been shown by Linford (7) and Snyder (16) to be around 28° C. Because of the inoculation technique employed in these experiments, it was deemed advisable to study the relation of temperature to growth

of the fungus in a nutrient solution having the same salt concentration and balance as that used for the growing plants. The 0.1H nutrient solution was made up with 2 percent dextrose, and 50 ml. of solution were added to each 250-ml. Erlenmeyer flask. Six inoculated flasks were placed at each of nine constant temperatures. At the end of 14 days, three flasks at each temperature were removed and the mycelial

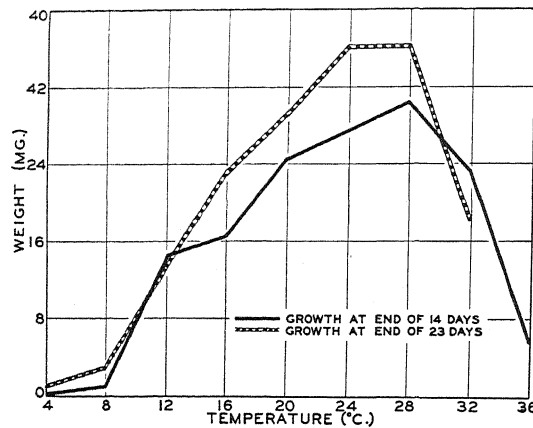


FIGURE 5.—Average weight of the mycelium of *F. oxysporum* f. *pisi* race 1 grown in 0.1H nutrient solution supplemented with 2 percent dextrose, at nine constant temperatures.

contents of each flask weighed; the other set of three was weighed at the end of 23 days. Prior to weighing, the mycelial growth was filtered, washed four times with distilled water, and the mats dried at 74° C. The pH of the filtrates at each temperature was not appreciably different from the original pH 4.35 in the sterile solution.

The results are shown graphically in figure 5. At the end of 14 days, 28° appeared to be the optimum temperature,

#### INFLUENCE OF SAND TEMPERATURE ON THE HOST-PARASITE RELATIONSHIP

It has been assumed that the influence of temperature on the development of fusarial wilts is primarily due to its direct effect upon the pathogen. Pea wilt has been a notable exception when plants were grown on infested soil, but the results just presented show that when soil is eliminated and infested quartz sand used the thermal relations do not differ from those of other vascular fusarioses. It cannot be definitely ascertained from the experiments reported herein whether or not the increased severity in disease development at 27° to 30° C. is due to the influence of these higher temperatures on the fungus, on the host, or on both. Nevertheless, certain observations on the development of the disease at the different sand temperatures do provide a better understanding of the relationship between host and parasite. Each plant in experiment 2 that had reached the complete-wilt stage was removed along with a corresponding control plant for further study. The smallest and the largest diameters of the first internode of each plant were measured. Root and nodal plantings were then made with each diseased plant. The first internode of the completely wilted Davis Perfection (susceptible) plants was significantly larger at each temperature than that of corresponding healthy



control plants (table 4). A comparison of the differences in diameter at the various temperatures showed this to be greatest at 21°,

TABLE 4.—Comparison of the diameters of the first internodes of wilted and healthy *Davis Perfection* (susceptible) plants at six different temperatures

Condition of plant	Average diameter (mm.) <sup>1</sup> at temperatures of—					
	15° C.	18° C.	21° C.	24° C.	27° C.	30° C.
Completely wilted .....	3.09	3.16	3.46	3.45	3.51	3.47
Healthy control .....	2.90	2.78	2.75	2.91	3.05	3.11
Difference .....	.19	.38	.71	.54	.45	.36

<sup>1</sup> Minimum significant difference in comparing (19:1) temperatures within the healthy or inoculated group or between healthy and wilted plants within any temperature is 0.18 mm.

which is near the optimum for the growth of the host (7). The wilted plants of the resistant variety showed an increase in diameter of the first internode which was of the same order as that found in the susceptible variety. When wilted resistant or susceptible plants were measured it was observed that the first and second internodes were fully rounded, in contrast to those of healthy plants which exhibited definite furrowing due to schizogenous lacunae in the cortical parenchyma. Microscopic examination of transections of healthy and wilted plants at corresponding locations in the first internode revealed that the lacunae had not developed or, at least, were greatly retarded in the latter group. It would appear, therefore, that this host reaction to fungus invasion is common to resistant and susceptible forms and its relation to temperature is a function of the influence of the latter on the host rather than on the pathogen.

The progress of the fungus through the axis of the plant was measured by root and nodal isolations as each particular plant reached complete wilting since this stage represented the ultimate expression of the interaction of host and parasite. The results (table 5) show

TABLE 5.—The influence of temperature on the advance of the pea-wilt organism in the stems of *Davis Perfection* (susceptible) plants at the complete-wilt stage

Sand temperature (°C.)	Total plants	Plants in which the fungus had reached the node indicated by numbering from the tip downward—										Average of nodes in the plant at the complete wilting stage
		Tip	1	2	3	4	5	6	7	8	9	
15.....	19	0	0	10.5	0	5.3	10.5	10.5	31.6	21.1	10.5	10.3
18.....	21	0	23.8	38.1	14.3	4.8	19.0	4.8	.....	.....	.....	8.6
21.....	22	31.8	31.8	27.3	9.1	.....	.....	.....	.....	.....	.....	8.1
24.....	21	61.9	19.0	19.0	.....	.....	.....	.....	.....	.....	.....	7.9
27.....	21	15.0	15.0	30.0	20.0	15.0	5.0	.....	.....	.....	.....	6.7
30.....	13	7.7	15.4	30.8	30.8	15.4	.....	.....	.....	.....	.....	6.5

that at 24° the fungus progressed to the tip in a greater percentage of plants than at any other temperature, while the closest approach to this situation occurred at 21°. The progress of the organism was definitely slower at 27° and 30° and still more so at 15° and 18°.

The average number of nodes per plant at the time of complete wilting was inversely proportional to the temperature. It is significant, however, that the organism reached the terminal node in fewer plants at 27° and 30° than it did at 24° and 21°, in spite of the fact that it had fewer nodes through which to travel and the additional fact that plants wilted at 27° and 30° were usually somewhat shorter than those that wilted at 24°. This would indicate that, insofar as this phase of host-parasite relationship is concerned, the influence of temperature on the pathogen is not strictly parallel to its effect on fungus growth.

The development of the disease beyond the appearance of initial symptoms was quite variable at the different temperatures, although the ultimate result was a withering and drying of the fully expanded leaves, followed by the death of the terminal bud. Once the initial symptoms appeared, wilting was most rapid at 27° and 30°, the uppermost leaves withered without any apparent change in color, and leaf abscission seldom occurred. At 21° the leaves of affected plants wilted more slowly and definite color changes preceded the death of each stipule and leaflet, while abscission of dead leaves occurred in most instances. Affected susceptible plants at 21° and at lower temperatures were very similar in appearance to resistant plants at 27° to 30° (fig. 6). Complete wilting in resistant plants seldom occurred at 15°, and when it did so it was characterized by a very slow necrosis and abscission of the leaflets until all but the terminal bud were dead.

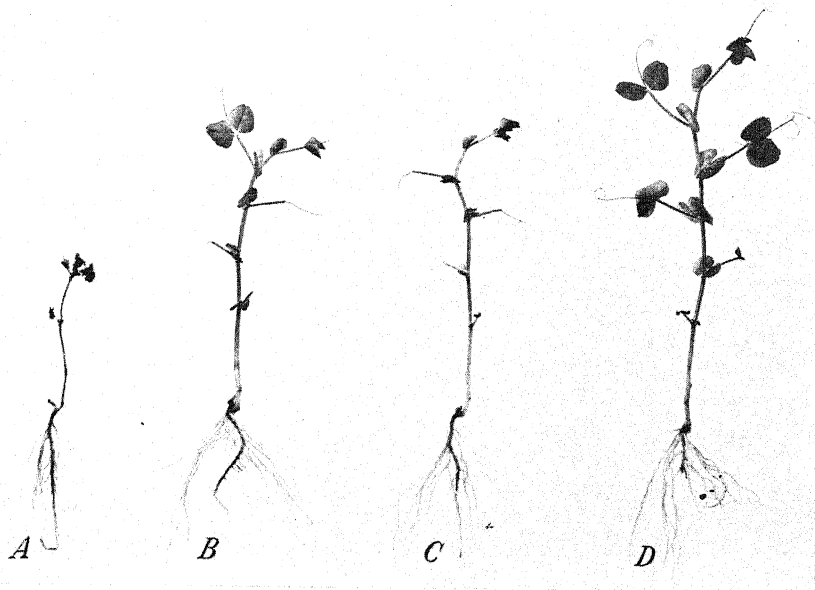


FIGURE 6.—A comparison of diseased pea plants of a susceptible and a resistant variety 23 days after planting, when grown at two different sand temperatures: A and B, Davis Perfection (susceptible) grown at 27° and 21°, respectively; C and D, Wisconsin Perfection (resistant) grown at 27° and 21°, respectively. All corresponding control plants approximated in size the plant on the extreme right.

An important characteristic of the disease in sand culture was that at 27° and 30°, which were the optima for the development of complete wilting, cortical necrosis of the roots was usually severe. Necrosis was progressively less prevalent as the temperature decreased. This is a phase of the disease which has seldom been mentioned by other workers who have used soil culture but is suggestive of a case described by Linford (9) on plants grown aseptically on inoculated soil. In view of the fact that the most rapid vascular invasion occurs at 24° and 21°, it may be that vascular invasion is responsible for the first appearance and most rapid advance of the disease in soil at 21°, while in sand where higher temperatures promote cortical invasion the higher optimum for complete wilting may be due in part to the injection of the cortical phase into the disease picture. In any case it is an interesting example of a shift by change in thermal environment from a more strictly vascular disease to one which is both vascular and cortical. It was not uncommon to find roots with extensive cortical decay in which healthy white lateral roots emerged through the necrotic area and served to delay the wilting of the above-ground stem and leaves. Such plants were found in the susceptible variety most commonly at 15° and in the resistant variety at 27° and 30° (fig. 7).

### NUTRITIONAL STUDIES

#### RELATION OF INCREASED NUTRIENT CONCENTRATION TO DISEASE DEVELOPMENT

The experiments on the relation of sand temperature to pea wilt established a higher optimum for disease development than that previously recorded for infested soil and indicated that resistance to the disease could be partially broken down, especially at 27° and 30°. Concomitant with the increased disease development at these temperatures was a pronounced cortical decay of the roots of both resistant and susceptible plants heretofore unassociated with pea wilt, except in one instance cited by Linford (9). Smith and Walker (14) showed that multiple concentrations of the nutrient solution described by Hoagland and Snyder (4) have a retarding effect on infection and severity of aphanomyces root rot of pea (*Aphanomyces euteiches* Drechs.) in which severe cortical decay of the roots and lower stem occurs. They obtained no infection at nutrient concentrations four and five times that used by Hoagland and Snyder (4).

In a preliminary experiment conducted during October 1940, at a sand temperature of 27° with two concentrations of the nutrient solution, 0.1H and 1H, it was observed that the appearance of complete wilting of the susceptible plants was delayed at the higher nutrient concentration. Likewise, the disease was regarded in its development and was much less severe in the resistant plants supplied with the higher concentration (fig. 8). Since observations indicated that cortical decay was quite characteristic of pea wilt in both susceptible and resistant varieties grown in sand at 27° and 30° C. and supplied with a dilute nutrient solution, the effect of higher nutrient concentrations on the development of pea wilt was studied. With but few exceptions, the method used was essentially the same as that in the sand-temperature work. The sand was held close to 21° and 27°, respectively, since these two temperatures effected marked differences

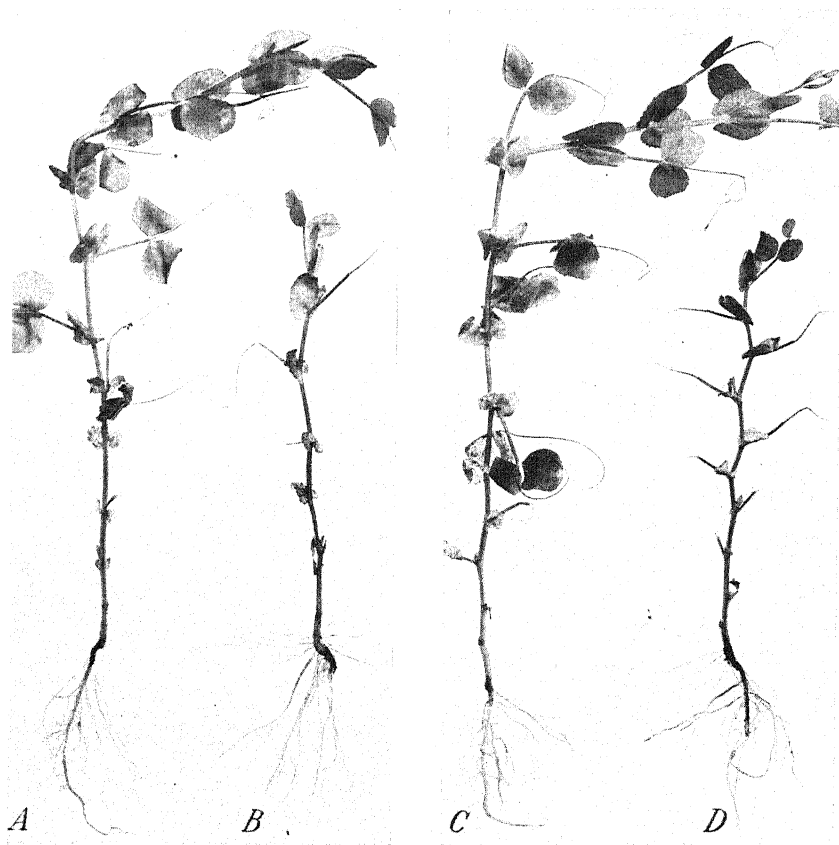


FIGURE 7.—Regeneration of the root system in peas following pronounced cortical decay produced by the wilt organism; this was found most commonly in wilt-susceptible peas grown at low sand temperatures and in wilt-resistant peas grown at high sand temperatures: A, Davis Perfection (susceptible) plant grown in uninoculated sand at 15° C.; B, Davis Perfection plant grown for 40 days in inoculated sand at 15°; C, Wisconsin Perfection (resistant) plant grown in uninoculated sand at 27°; D, Wisconsin Perfection plant grown for 40 days in inoculated sand at 27°.

in the development of the disease in both the resistant and the susceptible varieties. Two experiments were conducted, one from January 18 to March 5, the other from March 12 to May 4. The air temperature was maintained at 22° to 24° except on sunny days when it sometimes rose to 28° or slightly above for 4 to 6 hours. All inoculated crocks were run in triplicate, and the control crocks in duplicate. The progress of the disease was measured by the wilt index, except in the resistant variety in the second experiment, where the disease-development index was used because not enough complete wilting occurred to show the real effects among the nutrient concentrations.

Air-dry weights of the roots and stems of the healthy plants grown in the various concentrations of nutrient solution gave no indications of significant differences, except that, in general, the weights of the



stems of roots of plants growing at 21° were greater than those of the plants growing at 27°. Seedling emergence at 27° was 2 or 3 days earlier than at 21°. Plants growing in the 5H nutrient solution had smaller leaves, were somewhat shorter, and were distinctly darker green in color. No difference was observed in the size of the plants grown in the other nutrient concentrations, but the plants in the 0.1H solution possessed a lighter green color than those in the 1H, 3H, or 5H solutions.

It may be well to consider the effect of nutrients on disease development first at 21°, which is near the optimum for host development. The reaction at this temperature as a whole may then be compared



FIGURE 8.—Effect of nutrient concentration on the development of symptoms in Wisconsin Perfection (resistant) peas grown for 23 days at a sand temperature of 27° C: A, supplied with 0.1H solution; B, supplied with 1H solution. Note the leaf abscission and erect habit of stems in A.

with that at 27°, which is near the optimum for the pathogen and above the average of the range ordinarily encountered for any protracted period by peas in nature. The wilt indices of the susceptible plants are given in table 6. At 21° there was no significant difference

TABLE 6.—The effect of nutrient concentration on the development of wilt in Davis Perfection (susceptible) peas grown in artificially infested sand

Sand temperature (°C.)	Period of test	Wilt index at nutrient concentration—				Minimum significant difference (19:1)
		0.1H	1H	3H	5H	
21.....	Jan. 15–Mar. 5.....	25.6	26.6	22.3	23.8	(1)
21.....	Mar. 12–May 4.....	26.2	28.2	36.0	40.8	
27.....	Jan. 15–Mar. 5.....	18.6	18.6	15.1	13.3	3.65
27.....	Mar. 12–May 4.....	21.1	24.9	18.6	11.4	3.75

<sup>1</sup> Calculated *F* value indicated no significant differences among the 4 nutrient concentrations.

<sup>2</sup> 16.6 percent of the plants were diseased but not completely wilted at the end of the experiment.

between wilt indices at the four nutrient levels in the midwinter, short-day, low-light-intensity run. However, in the longer-day run with better light there was a definite increase in the wilt index and thus a slower development of disease with increase in nutrient concentration. At this temperature there were so few of the resistant plants which reached the complete-wilt stage that it was impossible to calculate comparable wilt indices and data are therefore not presented in the table. In both of the tests with resistant plants at 21°, however, the disease was most severe at 0.1H, less serious at 1H, and absent at 3H and 5H. At 21°, therefore, the increase in nutrient concentration had a depressive effect on disease except in the susceptible strain in midwinter when light was poor and days were short. Nutrition thus had the same general influence on wilt at this temperature as described for root rot (*Aphanomyces euteiches*) by Smith and Walker (14).

The picture at 27° was quite different. Severe cortical necrosis was evident on roots and lower stem internodes of susceptible and resistant plants at the two highest concentrations, 3H and 5H (fig. 9). There was a definite decrease in the wilt index and thus an increase in rate of wilt appearance with increase in nutrient level in susceptible plants (table 6) in both light periods at this temperature. This held true also for the short-day, low-light run with the resistant variety. It will be noted, however, that in the spring run with the susceptible variety (table 8) the disease developed significantly more slowly at 1H than at either 0.1H or 3H. This was even more pronounced in the resistant variety where the cortical decay was greatest at 3H and 5H. The result with the 0.1H and 1H concentrations coincided with that reported for the preliminary trial in October and illustrated for the resistant variety in figure 8. The plants at the 3H and 5H levels, however, showed increasingly severe necrosis and more rapid wilting. The resistant plants in the spring run at 21° and at 27° were removed at the end of the experiment and disease-development indices determined (fig. 10). The widely different reaction to nutrient concentration at the two temperatures is quite evident. At 21°, where vascular invasion was most prominent, there was a progressive decline in disease with increase in nutrient concentration. At 27°, on the other hand, where cortical decay entered the disease complex the rate of disease development declined when the nutrient level was raised up to 1H but increased sharply as the concentration rose above that level.

It is thus quite clear that temperature and nutrition each have an influence upon the type of disease reaction which prevails in sand culture. Generally speaking, under conditions of light and temperature favorable to the host (21°) a truer vascular disease prevails and increase in the concentration of the nutrient retards the pathologic development in the susceptible and particularly in the resistant variety. At the optimum temperature for the pathogen (27°) cortical decay of the root system is the predominant disease reaction while vascular invasion is secondary. Moreover, the combined effect is most severe in very weak (0.1H) and in very concentrated nutrient levels (3H and 5H) and least severe at an intermediate level (1H). This pronounced appearance of cortical invasion in a fusarial disease

ordinarily regarded as quite strictly vascular by a shift in environment tends to break down any definite line between cortical and vascular fusaria. It recalls, furthermore, the situation in certain other fusarial



FIGURE 9.—The effect of nutrient concentration on the development of cortical decay in sand held at 27° C. A, Davis Perfection (susceptible); B, Wisconsin Perfection (resistant). From left to right, plants grown in nutrient concentrations of 0.1H, 1H, 3H, and 5H, respectively.

wilts, such as that of watermelon, in which at certain stages in host development the disease is predominantly cortical and necrotic while at another stage it becomes chiefly vascular and hypoplastic (3, 24).

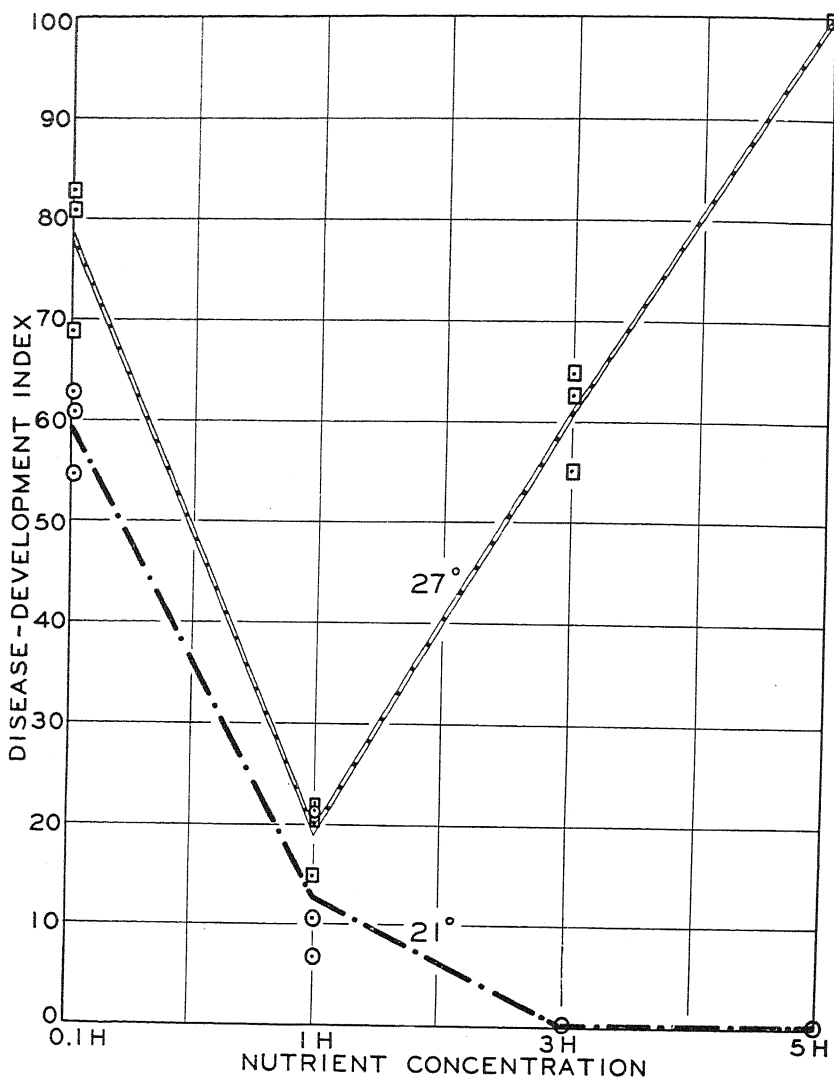


FIGURE 10.—The effect of four different nutrient concentrations on wilt development as shown by the disease-development index in Wisconsin Perfection (resistant) peas grown in sand held at 21° and 27° C.

#### PATHOLOGICAL ANATOMY

Previous histological examinations of the root systems of resistant plants grown under ideal conditions for soil infection failed to reveal the presence of the parasite in the stele (7, 21). Walker (21) found invasion in the outer cortical cells of resistant Alaska seedlings to be meager in contrast to that in susceptible roots when grown on soil-extract agar previously inoculated with the parasite. Thus, it was suggested that resistance to pea wilt in plants grown in soil or on



artificial media was expressed in the root-tip region. In this study, however, extensive cortical decay in both resistant and susceptible plants was observed. Nodal isolations of resistant plants affected with the disease indicated that the organism had progressed to as high as the sixth node. In view of these facts, histological examination of the affected resistant plants was undertaken to determine the location and extent of invasion by the parasite, and to observe any notable reaction between host and parasite that might differentiate the resistant from the susceptible plants.

Plants were removed from the sand, usually at the complete-wilt stage, and the desired sections were placed in either formol-acetic-alcohol or dioxan fixative. The staining procedure followed throughout was a combination of Mayer's haem-alum and orange G in clove oil (5, p. 78). Sections were cut 10 to 15 microns in thickness. The presence of the organism in the tissue was verified by plating out a part of the surface-sterilized tissue above and below the section placed in the fixative. Pathogenicity tests of some of these isolates covering a varied range of cultural characteristics indicated the same difference in symptom expression between resistant and susceptible plants as when the original culture was used; no marked difference in virulence between the isolates of resistant plants and the original culture was observed.

Penetration of the roots of both varieties was not limited to the root-tip region. In fact, many infected roots showed a sound meristematic region in contrast to that part behind the tip. Occasionally roots were found in which the upper extremity showed pronounced cortical decay, and the region between it and the growing point exhibited a complete collapse of tissue not accompanied by any water soaking. In many instances, especially at the higher temperatures, the taproot and many laterals were decidedly browned and water-soaked. Transsections of such roots showed pronounced cortical and stelar penetration (pl. 1). Inasmuch as the fungus was found in the phloem fibers it apparently encountered little difficulty in passing through the endodermal and pericyclic region of these roots (pl. 4, A). Sections of resistant roots were frequently found in which the parasite occurred in the outer rows of cortical cells (pl. 1, A and B). In such cases stelar penetration probably occurred at a lower level where cortical decay advanced to the stele. Hyphae were found in the xylem vessels of resistant plants as high as the first and second internodes (pl. 3, A). Nodal isolations indicated it had progressed still further in some plants.

No differences were found between varieties with respect to the enlargement of the lower internodes (pl. 2, A to D). This might be accounted for by an increase in cambial activity, especially in the plane of the polar bundles, which resulted in an increase in phloem and xylem tissue. Evidence of proliferation of xylem vessels and parenchyma in the region of the phloem fibers was also observed (pl. 4, B). These observations agree somewhat with those of Linford (10), wherein he noted a stimulation of xylem parenchyma by aerial-wound inoculation of both resistant and susceptible plants. The hypertrophy of xylem parenchyma that Linford (9) observed when he injected fungal filtrates through cut petioles of susceptible plants was not found in any of the diseased resistant plants. Another factor which might have accounted for the increased diameter of the wilt-

infected plants was the retardation in the development of the schizogenous lacunae.

Susceptible plants grown in sand at 27° usually exhibited a water soaking of the tissues in the first and second internodes when completely wilted. In such plants the fungus was found abundantly throughout the cortex and stele of these internodes (pl. 4, *C*). Such water soaking was rare at 24° and below, and microscopic examination of such tissues indicated a situation comparable to that described by Linford (7) in which the fungus was confined to the stele. The fungus was found only sparingly in the xylem of completely wilted susceptible plants grown at 15°, and the aerial symptoms of such plants closely resembled those of the completely wilted resistant plants grown at 27° and 30° (fig. 6). A transection of such a plant is compared in plate 2 with those of a completely wilted resistant plant at 27°, and corresponding healthy plants. The hyphae were found in only two xylem elements of the susceptible plants, but both these vessels were crowded (pl. 3, *B*). The fungus occurred in more than two xylem vessels in the resistant plant, but hyphal invasion of any one vessel was limited to one or two hyphae (pl. 3, *A*), and deposition of granular and gumlike material was more prevalent. Microscopic examination of other completely wilted plants grown at the higher sand temperatures indicated a greater frequency of occluded vessels in the resistant variety. Such occlusions were not necessarily associated with invaded cells, nor did they appear to hinder the fungus in all cases (pl. 3, *A*; pl. 4, *D*). The protoxylem vessels appeared to be more frequently occluded than other vessels. Occasionally, cells were found in which the hyphae appeared enlarged and somewhat granulated in contrast to the smaller and more normal-appearing hyphae in the invaded cells of susceptible plants.

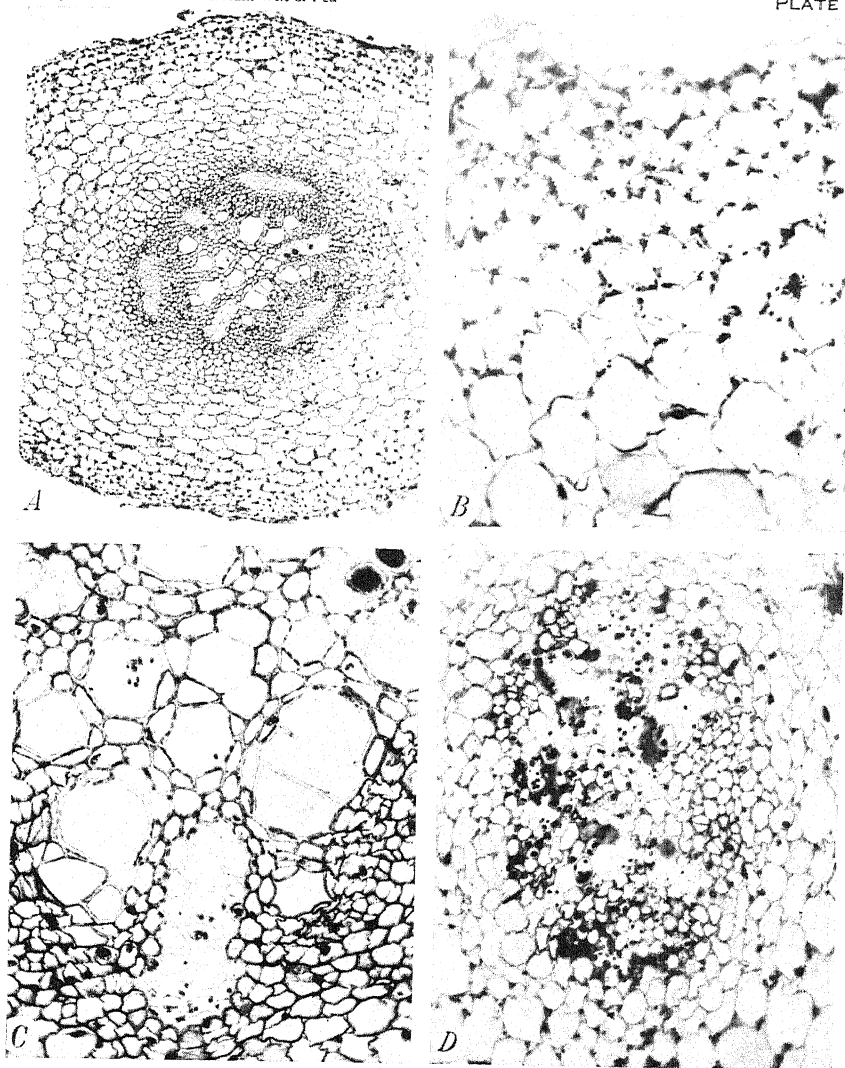
#### CROSS-INOCULATION STUDIES

The apparent break-down of wilt resistance in garden pea when plants were grown in inoculated sand supplied with a weak nutrient at high temperatures raised the question whether the host range of this organism could be extended under such conditions to hosts of other equally specialized vascular fusaria. Cross-inoculation experiments were therefore made with this organism, the cabbage yellows organism, and the tomato wilt organism, *F. oxysporum* Schlecht f. *lycopersici* (Sacc.) S. & H. (*F. bulbigenum* var. *lycopersici* (Brushi) Wr. & R.). This seemed especially pertinent in view of the recent work of Armstrong et al. (2) and Smith and Shaw (15), wherein definite wilting of supposedly immune hosts was obtained by inoculation with various vascular fusaria under special methods of nutrition and inoculation.

Two varieties of each host were used, one resistant and one susceptible to the respective organism which was known to be specialized to that host. The following varieties were included:

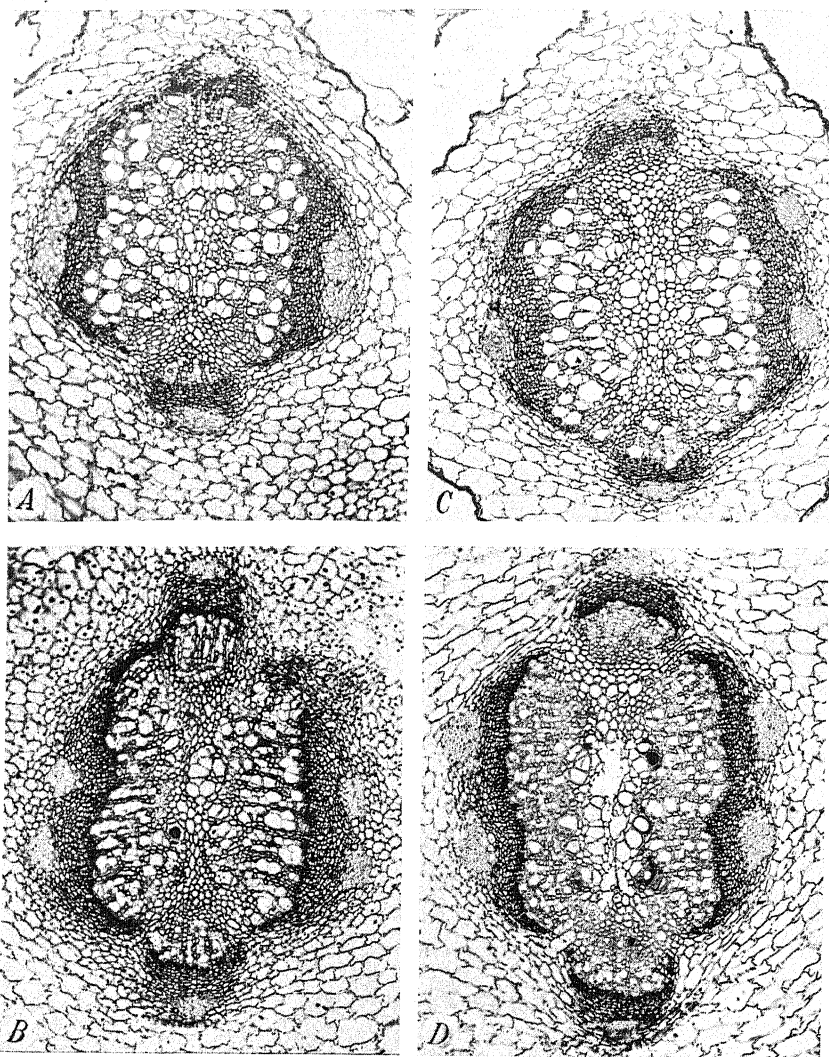
Host:	Resistant variety	Susceptible variety
Tomato.....	Red Currant <sup>1</sup> .....	John Baer.
Cabbage.....	Jersey Queen.....	Round Dutch.
Pea.....	Wisconsin Perfection.....	Davis Perfection.

<sup>1</sup> A highly resistant strain of this species (*Lycopersicon pimpinellifolium* Mill.) was used.



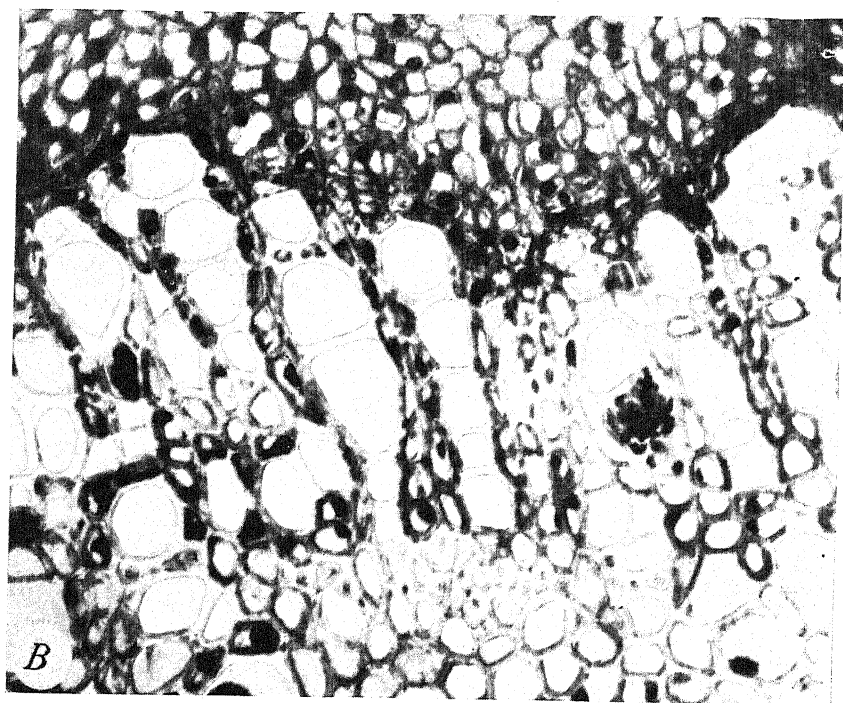
Transections of the roots of Wisconsin Perfection (resistant) peas. *A*, Penetration of the outer cortical cells and stele,  $\times 105$ ; *B*, enlarged portion of outer cortex in *A*, showing intra- and inter-cellular penetration,  $\times 433$ ; *C*, enlarged portion of stele in *A*, showing hyphae in metaxylem and protoxylem arm and also occlusions in some of the vessels,  $\times 433$ ; *D*, lateral root showing hyphae in cortex and stele and occlusions in some of the vessels,  $\times 433$ .



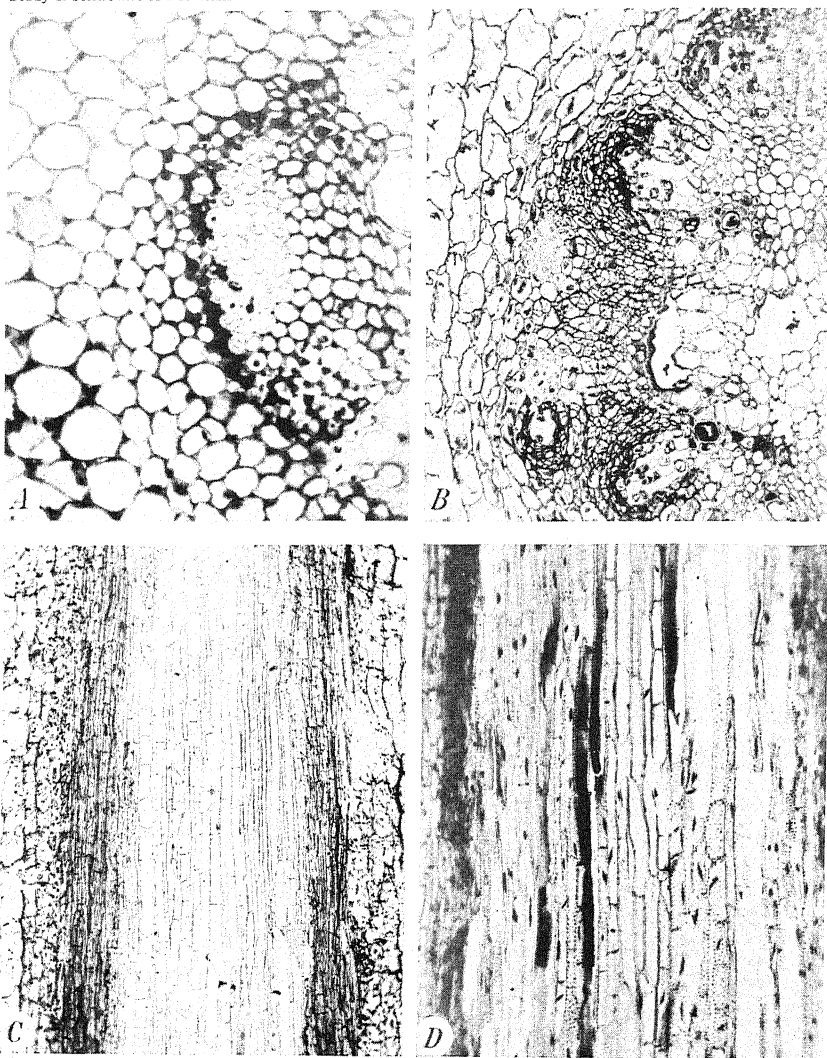


A to C, Transections of the first internodes of the plants illustrated in figure 7 to show similarity in host-parasite relations in resistant and susceptible plants. All  $\times 105$ . A, Healthy Davis Perfection pea (susceptible) grown at  $15^{\circ}$  C. sand temperature; note the development of schizogenous lacunae and normal cambial activity. B, Wilted Davis Perfection pea grown at  $15^{\circ}$ ; only two vessels are invaded but hyphae are abundant in those two; note the increased cambial activity and development of secondary xylem and phloem, especially in the plane of the polar bundles; the abundance of nuclei is the result of the development of adventitious roots at a lower level. See further enlargement in plate 3, B. C, Healthy Wisconsin Perfection (resistant) grown at  $27^{\circ}$ ; note similarity to A. D, Wilted Wisconsin Perfection grown at  $27^{\circ}$ ; compare with B for abnormal development of stelar tissues. Only one or two hyphae were contained in each of the invaded vessels; note the greater number of occluded and granulated cells. See further enlargement in plate 3, A.





*A*, Enlarged portion of stele of wilted Wisconsin Perfection (resistant) pea plant grown at 27°, illustrated in plate 2, *D*, showing rather sparse occurrence of the fungus and abundance of granular and gumlike depositions in the cells; note the two hyphal ends in the granulated cell to the right and the intercellular penetration of the hypha from a clear cell to an adjacent granulated cell. *B*, Enlarged portion of stele of wilted Davis Perfection (susceptible) pea plant grown at 15°, illustrated in plate 2, *B*, showing abundance of hyphae in the only two invaded vessels.



A, Transection of root of Wisconsin Perfection (resistant) plant showing penetration of hyphae in the cortex, phloem, and pericycle, as well as in some of the phloem fibers,  $\times 433$ . B, Transection of the first internode of a completely wilted Wisconsin Perfection plant at  $30^{\circ}$  C. showing an extreme reaction to infection; the fungus was present in the vessels, but not extensively. Note the cellular occlusions and the proliferation of vessels in the xylem region adjacent to the phloem fiber bundle,  $\times 433$ . C, Longitudinal section of the first internode of a completely wilted Davis Perfection (susceptible) plant at  $27^{\circ}$ , showing an abundance of hyphae in the cortex and cambial region, as well as in the stele; note the apparent absence of occlusions. D, Longitudinal section of the first internode of Wisconsin Perfection plant at  $27^{\circ}$ , showing considerable plugging of the vessels, especially in the protoxylem; compare with C.

The sand supporting the growth of these plants was held at 27° to 28° C., and supplied with the 0.1H concentration of nutrient solution. Seed of the pea varieties and transplants of the tomato and cabbage varieties were planted directly in the artificially infested sand. For the sake of compatibility with respect to any predisposing influence, the seed for the cabbage and tomato transplants was sown in sterilized sand supplied with the 0.1 H nutrient solution and maintained at approximately 28°. The crocks were planted with eight pea seeds and five to six transplants of cabbage and tomato. The inoculum was prepared as previously described for the pea wilt organism. The medium proved equally suitable for all three pathogens; the inocula consisted entirely of microconidia and mycelial fragments. All crocks were planted in duplicate. Notes were taken on the initial appearance of the disease, full symptom expression, and death of the plants.

The results indicated that all three pathogens were specific to their respective hosts under the conditions of this experiment. Davis Perfection (susceptible), inoculated with the pea wilt organism, succumbed to the disease 24 days after planting, while Wisconsin Perfection (resistant) exhibited the slow disease development characteristic of these plants under the conditions of low nutrient and high temperature. Plants of John Bear tomato (susceptible) inoculated with the tomato wilt fungus, and Round Dutch cabbage (susceptible) inoculated with the cabbage yellows organism, were dead at the end of 17 days. No visible disease symptoms occurred on Red Currant tomato in sand artificially infested with the tomato wilt organism. Jersey Queen cabbage (resistant), in the cabbage yellows sand, exhibited a severe expression of the atypical symptoms described by Walker and Smith (22); some plants died. Moreover, none of the hosts grown in sand infested with their respective nonspecific parasites was different in external appearance from the corresponding control plants.

Nodal plantings of the surviving plants indicated that the pathogens might have penetrated the nonspecific hosts even though they did not give rise to any symptom expression. This was especially characteristic of the resistant and susceptible varieties of pea. Plantings were made of the root, and of the second, fourth, and sixth nodes of the stem. The respective fungus was recovered from the second node of each plant and from the root and the third or fourth node of a few plants. The constant location of the organism in the second node suggested that penetration occurred through the region of cotyledonary attachment. The significance of these findings depends upon further studies and on re-cross inoculations with the fungi recovered from these plants. Nevertheless, the results indicate that of the three species only the pea wilt organism can produce symptoms in either the susceptible or the resistant variety of pea.

#### DISCUSSION

The fusarium wilt of pea has been of considerable biological interest, principally because of its several points of contrast with other related vascular fusarioses. Notable among these features have been the temperature relations, the symptoms, and the failure in attempts to



break down the inherent and clear-cut resistance of some varieties. The results presented in this paper show that, when the soil factor is eliminated by growing peas in sterile sand artificially infested with a pure culture of the pea wilt organism, disease development increases directly with the temperature of the sand, up to 27°. Weight of the mycelium of the fungus grown in the nutrient solution with added carbohydrate, is also greatest at that temperature. Thus the optimum temperature of pea wilt is essentially similar to that of tomato wilt, cabbage yellows, and near wilt of pea. The extreme departure from the disease-soil-temperature curve might have been explained on the basis of a new physiologic form or race of *F. ory-sporum* f. *pisi* race 1 were it not for the fact that a parallel series showed that in plants growing in soil disease development was neither as rapid nor as severe at 27°. Linford (7) suggested that the retardation in disease development at soil temperatures most favorable for the growth of the fungus be explained on the basis of resistance induced in the host by the high temperature. This view hardly seems tenable in the light of the present results, unless one assumes that the effects of temperature on plants in soil are remarkably different from those in sand. One possible explanation of the difference in reaction in soil and sand might be found in a study of the biological antagonism to the pea wilt organism in the soil at high temperatures. This suggestion does not seem too remote, when one considers the difficulty encountered by Walker and Snyder (23) in establishing the pea wilt fungus in certain types of soils, in contrast to the ease with which the cabbage yellows organism was established.

When plants were grown in sand held at 21° and supplied with nutrients at different concentration levels during that time of year when the periods of sunlight were fairly long, it was observed that the severity of disease development in susceptible plants decreased with a rise in the nutrient concentration level. Under the same conditions, the most severe development occurred in the resistant variety at 0.1H, very little occurred at 1H, and no evidence of disease was noted at 3H and 5H. These results are in agreement with those obtained by Smith and Walker (14) in a study of the relation of nutrient concentration to infection and severity of aphanomyces root rot of pea. During the middle of the winter, when the days were short and cloudy, the resistant plants reacted similarly but no significant differences were obtained in the wilt indices of susceptible plants at the various concentration levels. No explanation of this discrepancy can be given at the present time, but it would appear that a study of the effect of the duration and intensity of light on the development of the disease under the conditions of these experiments might yield important results.

When plants were grown at 27°, the results were altogether different. Instead of very little disease development occurring at the 5H nutrient, the disease was most severe at that level and no difference was observed between the susceptible and resistant varieties. Resistant plants succumbing under these conditions resembled the susceptible plants in the 0.1H nutrient level at 27°, except that the plants reached the complete-wilt stage in a much shorter time at the highest level. This occurred in both experiments, regardless of any possible effect of the light period. Cortical decay was very severe in the plants



at the high nutrient concentration level, and the abundance of fungal growth and its accompanying toxic action undoubtedly contributed to the rapid wilting.

It will be noted that Linford (9) observed extensive cortical decay in susceptible plants grown in wilt-infested soil under pure culture conditions. Moreover, the artificially infested sand used in the present investigation very closely approximated a pure culture condition, and in addition, the biological factors of the soil were eliminated. Anderson and Walker (1) also observed extensive cortical decay in the root hypocotyl of the homozygous resistant cabbage, Wisconsin Ballhead, when grown in yellows-infested soil at high temperatures. Thus it is to be seen that two diseases, both considered to be strictly vascular and controlled by clear-cut resistance under natural conditions, can be changed in their host-parasite relations by altering the conditions under which disease development occurs. Other fusarial wilts, such as watermelon wilt, muskmelon wilt, and fusarium yellows of celery, considered to be less strictly vascular wilts, develop a cortical decay in the roots under less drastic changes in environmental conditions. Nelson, Coons, and Cochran (11) observed extensive decay of celery roots in the field, especially when temperatures were quite high, whereas cortical decay and seedling rot occurred in plants affected with watermelon and muskmelon wilt at relatively low soil temperatures (6, 12, 13, 24).

The thermal and nutritional relations of the host, the parasite, and the interaction of the two may be seen to have an influence on the type and extent of disease development. The extension of the pathogen in the vascular system of the host was greatest at 24° and temperature and next most rapid at 21°. Swelling of the first stem internode following infection, due to vascular proliferation and to the lack of schizogenous lacunae, was greatest at 21°. These phases of the disease reach their peak, therefore, at temperatures close to that of optimum growth of the host. Cortical decay was greatest at 27° and 30°, points beyond that of best growth of the host but coincident with that for most rapid development of the pathogen. If this is compared with the thermal relations of watermelon wilt (12, 13, 24) it will be seen that the necrotic phase in the latter occurs chiefly at the lower temperatures which are least favorable to the host, while the typical wilt symptoms and vascular invasion come at the higher temperatures which are favorable to the host as well as to the parasite. It is thus very apparent that the influence of temperature on development of vascular wilts is not a simple one. The influence of nutrition throws much light on this question and furthermore this is shown to be an important interacting factor. It is clear that with light and temperature conditions favorable to growth of the pea plant an increase in available nutrients results in suppression of disease development in both resistant and susceptible varieties. However, at a temperature most favorable to the pathogen and less favorable to the host high nutrient levels have an opposite effect in that they promote cortical necrosis and rapid wilt development.

All attempts by other workers to break down resistance to pea wilt by such practices as severe root pruning and wounding of established plants or transplants has failed (7, 9, 19, 21). Most of this work was done in soil or on soil-extract agar at about 21° C., which was con-

sidered to be the optimum temperature for disease development. Occasionally evidence of slight invasion of the root tips of resistant plants was found, but, in contrast to the susceptible plants where the organism progressed from the root tip through to the stele and involved very little cortical decay, further progress ceased at this point. As a result, resistance was suggested to be located in the undifferentiated tissues of the young roots. In this investigation it was found that, as in cabbage yellows (1, 22), resistance to pea wilt was not retained at high sand temperatures. Resistant plants developed above-ground symptoms at high temperatures quite similar to susceptible plants at the lower sand temperatures of 15° and 18°. Coincident with slow wilt development in susceptible plants at 15° there was a very sparse invasion of the vascular elements and no cortical decay. In resistant plants grown at sand temperatures of 27° and 30° hyphal accumulation was also very sparse, but the internal reaction in such plants was not exactly the same as that of susceptible plants at 15°. In susceptible plants growing at 27° in sand supplied with either the 0.1H or the 5H nutrient solution and in the resistant plants supplied with the 5H at the same temperature very rapid wilting usually occurred. In such plants wilting simulated that in the severe development of tomato wilt. Cortical decay was extensive throughout the root system and the lower internodes, but wilting did not result from a collapse of the water-soaked lower internodes, since they were the last to collapse, indicating that the rapid wilting from the tip down might have been due to an abundance of toxin produced by the fungus. Hyphae were found in abundance throughout the cortex and stele of these lower internodes, but further extension through the xylem and cortex seemed to stop rather abruptly. From these observations it would appear that the severity of disease expression was related to the amount of hyphal invasion. Additional evidence in support of this suggestion was brought out in the nodal isolations of completely wilted plants grown at the various sand temperatures.

In addition to the very close similarity in symptoms between the susceptible plants at the low temperature and resistant plants at the high temperatures under conditions of low nutrition, microscopic examinations of the epicotyledonary tissues indicated that hyphal invasion of the stele was very sparse. Evidences of disturbances within the susceptible host were only rarely found, even in those rapidly wilted at the higher temperatures. Depositions of gumlike and granular materials were quite common in the stelar tissues of the epicotyl of resistant plants, and occurred to a lesser extent in the root. Almost all of the invaded vessels in resistant plants contained only one or two hyphal strands in the epicotyledonary tissues, in contrast to the root tissues where invasion of both cortex and stele was quite abundant. This fact, together with the pronounced host reaction, would seem to indicate that, even though well established within the root system, the organism progressed with difficulty through the resistant stem tissue. The evidence obtained from these studies seems to indicate that resistance to pea wilt is not necessarily located in the undifferentiated tissues of the root, for even though the organism invades the entire root system, it is still hindered in its progress through the stelar tissues of the stem. The nature of this hindrance would in a large part determine the nature of resistance.

The results obtained in the cross-inoculation studies validate those obtained with respect to the break-down in resistance to pea wilt under conditions of very low nutrition. Cross inoculations of susceptible and resistant varieties of tomato, cabbage, and pea with their respective fusarial wilt pathogens failed to alter their specificity, although the nodal isolation of these plants indicated that the fungi had penetrated the nonspecific hosts. The extent of penetration, as measured by the nodal isolations, was no greater in the resistant pea variety inoculated with the pea wilt organism than in the same variety inoculated with the cabbage yellows or tomato wilt pathogens. The presence of the pea wilt fungus in the plant, however, produced definite wilt symptoms; whereas no symptoms were observed on the same variety infected with the other two pathogens. Armstrong et al. (2) studied cross inoculations of various crop plants with their respective fusarial wilt pathogens and found that some were successful with respect to symptoms; others were not, although in many cases the fungus was recovered. The three pathogens used in the studies made by the writers were not included in Armstrong's work. These results, although not conclusive, suggest the possibility that a given fusarium pathogen may become established in a nonspecific host without producing symptoms, either because of its inability to produce a wilt-inducing toxin in that particular plant or because of the ability of the plant to tolerate any toxin produced.

#### SUMMARY

The investigations comprised a study of the influence of temperature and nutrition upon the development of fusarium wilt of pea (*Fusarium oxysporum* f. *pisi* race 1) in a susceptible and a resistant variety.

Under conditions of controlled nutrition and temperature in sand artificially infested by means of a suspension of microconidia and hyphal fragments, the optimum temperature for disease development was found to be 27° and 30° C. instead of 21° as previously observed in infested soil. Air temperature had relatively little influence on disease development. The fungus grew best at 28° when cultured in the same nutrient solution used in the sand culture of the host to which carbohydrate had been added.

The severity of disease development in the susceptible plants appeared to be directly proportional to the temperature, within the range studied. Symptoms varied from a very slow wilt characterized by leaf necrosis and abscission at the low sand temperature to a very rapid wilting at the highest temperature studied. Cortical decay occurred to some extent at all temperatures but was most severe and involved the lower internodes only at the optimum temperature. The greatest progress of the fungus up the stem occurred in those plants growing at the 24° and 21° sand temperatures. The lower internodes of diseased plants were significantly greater in diameter than the corresponding control plants.

The development of the disease in the homozygous resistant plants varied from off color and very slight incurving of the lower stipules and leaflets at the low temperatures and low nutrient concentration to severe wilting at the very highest concentration and optimum temperature. At high temperatures and low nutrient concentration diseased

resistant plants were similar to diseased susceptible plants at the low temperature.

The disease development in resistant and susceptible plants at various concentrations of the nutrient solution was found to depend upon the sand temperatures. Four nutrient solutions, differing only in total salt concentration and designated, in order of concentration, as 0.1H, 1H, 3H, and 5H were used. At 21°, the time required for the appearance of complete wilting in susceptible plants varied directly with the concentration of the nutrient solution; in resistant plants disease development was most severe in the 0.1H solution, and none occurred at the higher concentrations. At 27°, disease development was most severe in the 5H solutions, and no differences in symptoms were observed between susceptible and resistant plants. The least amount of disease development in both resistant and susceptible plants occurred in the 1H solution; that at the 3H level was quite variable. In the 0.1H solution, resistant plants developed a very severe case of slow wilting, while susceptible plants approached the rapid wilting occurrent in the 5H solution.

No differences were found in the growth rates of the fungus on agar media made from the nutrient solutions employed in the sand culture with added carbohydrate that could account for the differences observed in the disease development of plants growing in the different solutions at any given temperature.

Microscopical examination of diseased resistant plants indicated extensive cortical and stelar penetration of the roots. Stelar penetration was observed in sections taken from the first and second internodes; nodal isolations indicated still higher advances. More pronounced host reaction to the fungus, in the form of granular and gum-like depositions and scarcity of hyphal strands, was observed in resistant plants at the high temperatures than in susceptible plants at either high or low temperature.

Cross inoculations of susceptible and resistant varieties of tomato, pea, and cabbage with their respective fusarial wilt pathogens under the conditions of high temperatures and low nutrient sand culture indicated that the break-down in pea wilt resistance could be accomplished only with the pea wilt organism.

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# INFLUENCE OF HEREDITY AND OTHER FACTORS ON 180-DAY WEIGHT IN POLAND CHINA SWINE<sup>1</sup>

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## INTRODUCTION

Rate of gain to market weight is one of the important factors in economical swine production, since rapid gains cause lower maintenance costs and presumably indicate more vitality and a higher degree of health. If breeders are to make the best use of selection and various breeding systems in improving rate of gain in their herds, it is important to know something of the heritability of individual differences in this character. This paper presents a study of the effect of certain environmental factors on weight at 180 days of age and estimates of what part of the variance is hereditary as determined by the resemblance between certain kinds of relatives.

Few quantitative studies have been made of the influence of heredity on growth rate in swine. Lush and his coworkers (5)<sup>2</sup> concluded that 6 percent of the total variance in birth weight was attributable to differences in the heredity of the pigs, 47 percent to the environment common to litter mates, and 47 percent to the environment not common to litter mates. A part of the environmental effects common to litter mates may of course have been due to hereditary characteristics of their dams. From the correlations between certain relatives, Bywaters (1) concluded that one-fifth of the variance in weaning weight was determined by the heredity of the pig. Lush (4) studied Danish testing-station data and concluded that one-fifth of the variance in daily gain while the pigs were on test could be attributed to additive genetic effects. Smith and Donald (6) observed the correlation between the growth rates of litter mates to be 0.3 and 0.5 for litter sizes of 8 and 9, respectively. They concluded that at least one-fifth of the individual variance in growth rate was due to additive genetic factors.

## MATERIAL AND METHODS

The data for the present investigation were taken from an inbreeding experiment with Poland China swine begun at the Iowa Agricultural

<sup>1</sup> Received for publication October 23, 1941. Journal Paper No. J-917 of the Iowa Agricultural Experiment Station. Project No. 32. The studies reported here were made under the guidance of Dr. Jay L. Lush. The most recent of the data were collected as part of the cooperative work of the U. S. Regional Swine Breeding Laboratory.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 263.

Experiment Station in 1930. A brief history of the foundation animals has been given by Bywaters (1). The foundation stock consisted of 27 sows and 3 boars purchased from various breeders. Some of the sows were already bred when purchased and through these the offspring of 3 more boars were included in the foundation stock. No outside blood was introduced into the herd after 1930. Most of the foundation animals were not inbred. The 12 which were inbred had inbreeding coefficients of less than 4 percent. All inbreeding coefficients were computed on pedigrees traced to 1925 as a base date.

Each season the general breeding plan was to use four boars and to allot the sows so that each boar was mated to a group approximately equal to the other groups in average age, past productiveness, type, and in relationship to the boar to which they were bred. In the fall of 1937 the herd became a part of the Regional Swine Breeding Laboratory herd, and the original four-sire herd was split into several noninterbreeding lines within which there were different rates of inbreeding. However, the present study includes only one pig crop after the separation of lines, and the lines had not yet diverged far enough from the original four-sire herd to be materially different from each other.

During the gestation and suckling period all sows were fed and managed in the same manner. Pigs were weaned at 60 days in the first years of the experiment, but beginning with the fall of 1937, the weaning age was advanced to 56 days. After weaning, the pigs were fed good rations and were managed in accordance with good practices, which should have permitted them to show their capabilities. Although the pigs were not all fed and handled in exactly the same way, the different treatments were all reasonably good and the average 180-day weight of the groups on different treatments varied little. When the data were corrected for such treatment differences as did exist, the analyses gave results in such close agreement with the uncorrected data that corrections for treatment differences were not considered necessary for the present analysis.

The data covered 5 spring farrowing seasons (from 1934 through 1938) and 3 fall farrowing seasons (1934, 1935, and 1937). The 1,394 pigs produced during this period were in 267 litters, which were out of 151 sows and by 23 boars.

Weight at 180 days, rather than at some other age, was selected as the measure of gain in this study because at this age the faster growing pigs should be approaching a desirable market weight of 200 to 225 pounds. For pigs weighed a few days older or younger than 180 days, the 180-day weight was estimated by multiplying the actual weight by  $\frac{(180-60)}{(\text{actual age}-60)}$ . This adjustment was derived from the intercepts of straight lines fitted to several growth curves as was suggested by Bywaters and Willham (2). A later study<sup>3</sup> revealed that an intercept of 60 days gave more consistent results than the intercept of 65 days suggested by Bywaters and Willham. The data were analyzed chiefly by means of correlation, regression, analysis of variance, and covariance (7).

<sup>3</sup> WHATLEY, J. A., JR. A METHOD FOR COMPARING THE GROWTH RATES OF PIGS WEIGHED AT DIFFERENT AGES. Unpublished thesis. Iowa State College, Ames, Iowa. 1937.



## INFLUENCE OF DIFFERENT FACTORS AFFECTING 180-DAY WEIGHT

## INFLUENCE OF INBREEDING

The average inbreeding of all pigs was 14.7 percent, which is slightly higher than the inbreeding resulting from one generation of half-brother and sister mating (12.5 percent). The average weight and average inbreeding for each farrowing season are plotted in figure 1.

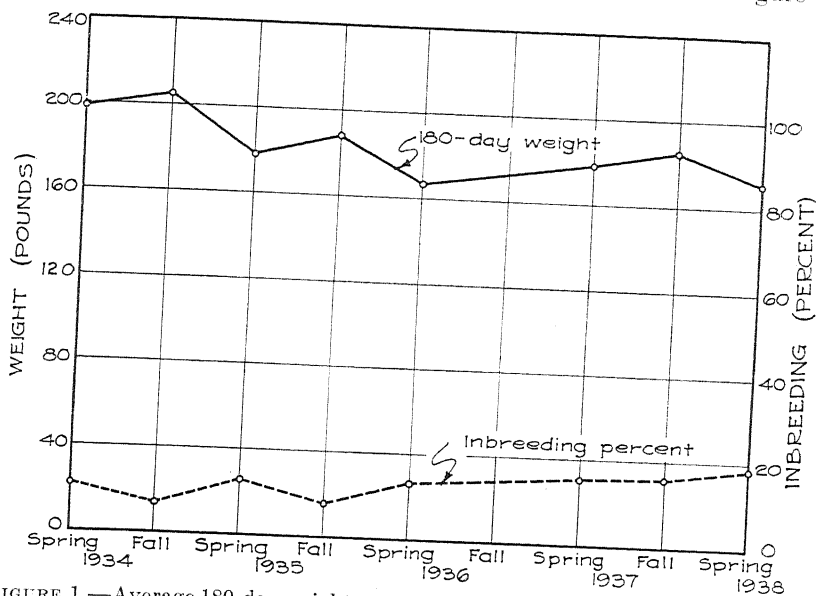


FIGURE 1.—Average 180-day weight and percent of inbreeding by season and year.

The average weight decreased slightly during the period studied, while the inbreeding gradually increased. The inbreeding was low in the fall seasons of 1934 and 1935, because at that time the policy was to produce fall pigs only from sows at least 2½ years old, and these naturally were less closely related to the boars than were sows bred for spring farrowing, about half of which were gilts. The average inbreeding of the sires and dams of the pigs was 9.3 percent.

A correlation of  $-0.17 \pm 0.03$ \* was observed between the inbreeding and the 180-day weight of pigs born in the same farrowing season. The corresponding regression coefficient was 0.76 pound decline in 180-day weight for each 1 percent increase in inbreeding. Table 1 shows the average 180-day weights when grouped according to the percentage of inbreeding. Except in the last two classes there was a noticeable decrease in weight with an increase in inbreeding. The sharp rise in weight in the two classes with the highest inbreeding may have been due to sampling errors, since the number of pigs in these classes was small.

\* The figures after the  $\pm$  signs in this paper are standard errors. For correlations they were computed according to the formula  $\frac{1-r^2}{\sqrt{n-2}}$ , which seemed a good enough approximation for correlations no larger than these.

When each litter was treated as a unit (rather than each pig as above), the correlation between 180-day weight and inbreeding was  $-0.19 \pm 0.06$ . The effect of the dam's inbreeding was negligible in these data, but as this mild inbreeding system was really just getting well under way, none of the dams were highly inbred.

#### INFLUENCE OF SEASON AND OF YEAR

The average 180-day weight of all pigs was 180.3 pounds and the standard deviation was 31.3 pounds. Figure 2 shows the distribution of the weights of the 1,394 pigs.

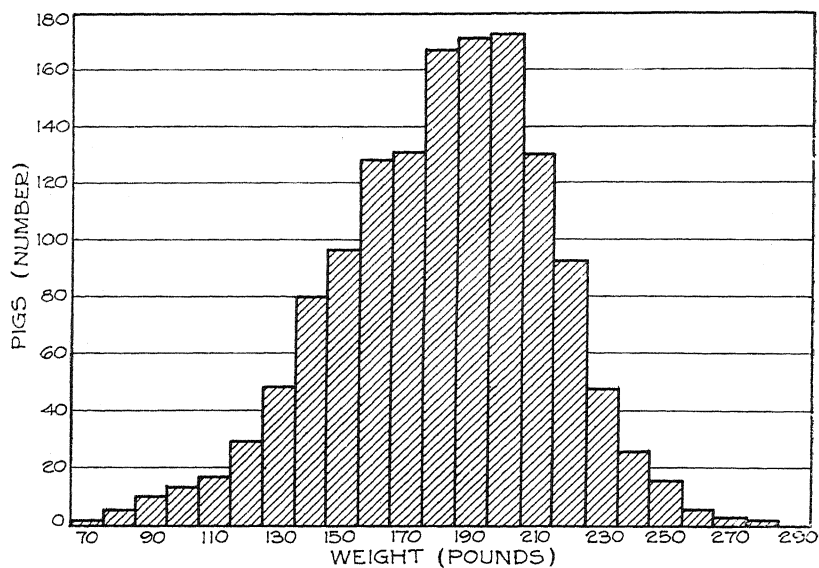


FIGURE 2.—Distribution of 180-day weights of 1,394 pigs.

The average weight of the 1,190 spring-farrowed pigs was 178 pounds as compared with an average weight of 194 pounds for the 204 fall-farrowed pigs. The difference of 16 pounds was highly significant statistically and is large enough to be economically important. Its importance as compared to other sources of variation is shown by the fact that the mean variance within pairs of pigs, one of which was born in the fall and the other in the spring, was 12.8 percent larger than the mean variance between pigs born in the same season.

TABLE 1.—Regression of 180-day weight on percent of inbreeding

Inbreeding (percent)	Litters	Pigs	180-day weight	Inbreeding (percent)	Litters	Pigs	180-day weight
	Number	Number	Pounds		Number	Number	Pounds
3.....	20	109	199	33.....	20	92	159
9.....	88	473	187	39.....	4	14	192
15.....	77	398	177	45.....	1	4	178
21.....	46	243	177				
27.....	11	61	166	Total or average...	267	1,394	180

In measuring the effect of year-to-year changes on 180-day weight only spring-farrowed pigs were considered, since fall pigs were not produced in all years. Among the spring-farrowed pigs the mean square between years was significantly larger than the mean square between litters farrowed in the same year. The variance between pigs born in different years averaged 11 percent higher than that between pigs born in the same year. This expresses quantitatively, for comparison with other sources of variance, the importance of the year-to-year variations in mean weights which are shown graphically in figure 1. It seems likely that a part of this year-to-year variation results from the time trend toward more intense inbreeding.

#### INFLUENCE OF DAM'S AGE

The influence of age of dam on the weight of pigs at 180 days was not statistically significant, although the figures in table 2, if accepted at face value, do indicate that pigs from older dams were slightly heavier. The superior nursing ability of the older dams might account for this slight difference (if more extensive data prove its reality), but also the older dams were selected in part on the performance of their previous litters and this might account for some such difference.

TABLE 2.—Average 180-day weights of spring and fall pigs from dams of different ages

Spring			Fall		
Age of dam (years)	Pigs	Average 180-day weight	Age of dam (years)	Pigs	Average 180-day weight
	<i>Number</i>	<i>Pounds</i>		<i>Number</i>	<i>Pounds</i>
1.....	624	174	1.5.....	35	192
2.....	293	180	2.5.....	99	195
3.....	136	182	3.5.....	27	189
4.....	77	189	4.5.....	27	193
5.....	49	182	5.5.....	16	206
6.....	11	164			
Total.....	1,190	178		204	194

#### INFLUENCE OF SEX

Gilts weighed about 4 percent less than barrows and boars at 180 days, and this difference was highly significant statistically. The average weight of the 694 males (620 barrows and 74 boars) was 184 pounds and the average weight of the 700 gilts was 177 pounds. The variance between pigs of unlike sex was about 2 percent larger than the variance between pigs of the same sex.

#### INFLUENCE OF BIRTH WEIGHT AND WEANING WEIGHT

The usefulness of birth weight and weaning weight for predicting weight at 180 days was studied with intralitter correlations between the three weights. The intralitter basis was used in order to eliminate the effects of environmental conditions which may have varied from litter to litter as well as from season to season. The correlations were:

- 0.55 ± 0.02 between 180-day weight (*W*) and weaning weight (*Y*)
- 0.43 ± 0.02 between 180-day weight (*W*) and birth weight (*Z*)
- 0.48 ± 0.02 between weaning weight (*Y*) and birth weight (*Z*)

The multiple correlation coefficient with 180-day weight dependent on birth weight and weaning weight was 0.58, as compared with a simple correlation coefficient of 0.55 between 180-day weight and weaning weight. Birth weight added to the multiple correlation only a little information that was not already included in the weaning weight. The standard net regression coefficients (betas) were  $+0.45$  for weaning weight and  $+0.21$  for birth weight or, in terms of a score card, weaning weight is twice as useful as birth weight in predicting weight at 180 days. The part of variance in 180-day weight that would have disappeared if both birth weight and weaning weight had been held constant was 33.6 percent. Weaning weight accounted for 20.2 of this, birth weight 4.3, and the joint effects of birth weight and weaning weight 9.1.

#### STATISTICAL STUDY OF HERITABILITY

##### REGRESSION OF THE VARIANCE IN 180-DAY WEIGHTS ON THE GENETIC RELATIONSHIP BETWEEN PIGS

Related individuals are likely to have received some of the same genes from common ancestors and for that reason they may be expected to be more alike in their hereditary traits than are unrelated individuals. The variance remaining within a pair whose members are genetically identical is that due to environment. Wright's (10) coefficient of relationship measures the probable genetic likeness between individuals related in various ways by descent. By computing the regression of the variance between pigs on their relationship to each other in an actual population of pigs showing a considerable range of relationships to each other, it is possible to estimate how much variance among unrelated pigs in this population was caused by differences in their heredity and how much by differences in their environment.

TABLE 3.—*Illustration of the intralot pairing of pigs for computing the regression of the variance in 180-day weight on the genetic relationship between pigs*

Pigs paired	Relationship coefficient	Variance in 180-day weight
		<i>Pounds</i>
185 and 145.....	0.206	128
323 and 252.....	.192	288
94 and 200.....	.451	32
121 and 175.....	.096	180
82 and (121+175).....	.206	140
(94+200) and (323+252).....	.266	1,936

In arranging the data for this part of the analysis, only pigs that were kept in the same lot were compared. This was done in order to eliminate as much as possible of the variation due to controllable environment. No pig was compared with a litter mate. This restriction on the pairing was made to insure that differences between dams would exist in every comparison. Thus the larger common environment of litter mates than of nonlitter mates was not permitted to affect the regression. One of the pairs in each lot consisted of the two most closely related pigs and another consisted of the two least closely related pigs. The remaining individual pigs were paired at random. If odd pigs left over from pairing were present, they were matched with one of the pairs, then one pair was matched with another pair. Groups of three or more could have been paired with each other to use all the



information about the intralot variance in 180-day weight; however, the extra information gained by including these complex comparisons was not thought to be worth the extra computation necessary. Table 3 illustrates this pairing in one lot of nine pigs. In the first four lines the relationship coefficient is simply Wright's (10) coefficient of relationship between two individuals related by descent. Pigs 121 and 175 were paired because they were the least closely related pair in this lot, while pigs 94 and 200 were the most closely related. In the fifth line the relationship is the probable correlation between the genotype of pig 82 and an average of the genotypes of pigs 121 and 175, while the variance similarly is that corresponding to the one degree of freedom between the weight of pig 82 and the average weight of pigs 121 and 175.

Figure 3 is a path-coefficient diagram showing how the correlation

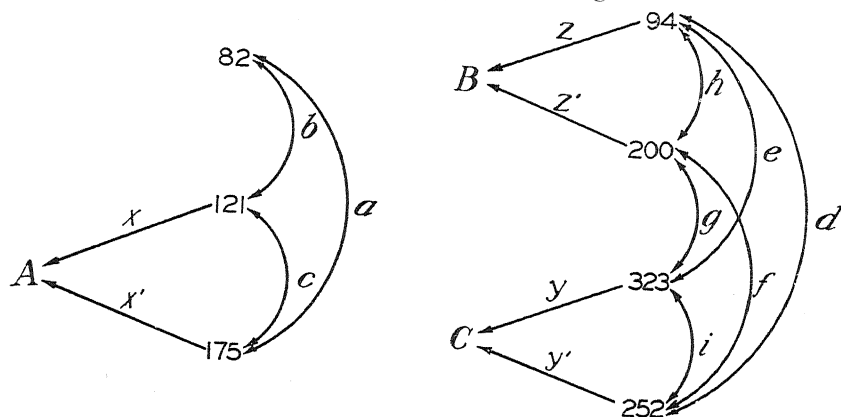


FIGURE 3.—Path-coefficient diagram of the biometric relations involved in the genotypic correlation between one pig and the average of two others, and between the averages of two pairs of pigs: Correlation (left) of pig 82 with *A*, the average of pigs 121 and 175; correlation (right) of *B*, the average of pigs 94 and 200, with *C*, the average of pigs 323 and 252.

between the genotype of pig 82 and *A* was completed.  $r_{82 \times A} = bx + ax'$  which  $= \frac{a+b}{\sqrt{2(1+c)}}$  if  $x=x'$ . Now  $x = \frac{\sigma_{121}}{\sigma_A}$  and  $x' = \frac{\sigma_{175}}{\sigma_A}$ . These need not be exactly identical but will not vary much since  $\sigma_{121} = \sqrt{1+F_{121}}$  times the genetic standard deviation in the population at the base date to which pedigrees were traced for computing relationships, and  $\sigma_{175} = \sqrt{1+F_{175}}$  times the same standard deviation. The range in inbreeding coefficients was not enough to cause  $x$  and  $x'$  to be very different in most pairs in this population. For convenience  $r_{82 \times A}$  was computed by the above formula which required only the substitution of  $a=0.113$ ,  $b=0.193$ , and  $c=0.096$  to yield 0.206 in this case.

In a similar way the correlation between genotypes in a comparison of two pigs with two others (fig. 3, right) is as follows:

*B* = the average genotype of pigs 94 and 200, and *C* is the average genotype of pigs 323 and 252.

$$r_{BC} = zey + zdy' + z'gy + z'fy' =$$

the sum of four covariances of 94 and 200 with 323 and 252

$$\sigma_B \sigma_C$$

It seems approximately correct to assume that  $z=z'$  and  $y=y'$  but not that  $z=y$ , since  $i$  and  $h$  may be quite different. Some pairs were selected for the very reason that they were slightly correlated genotypically and others were chosen because they were highly correlated.

Therefore  $r_{BC}$  was computed as equal to  $\frac{d+e+f+g}{2\sqrt{(1+h)(1+i)}}$  and in figure 3 (right), where  $d=0.128$ ,  $e=0.132$ ,  $f=0.256$ ,  $g=0.183$ ,  $h=0.451$ , and  $i=0.192$ ,  $r_{BC}=0.266$ , which is shown in the bottom line of table 3. The figures in the last column of table 3 are mean squares corresponding to each individual degree of freedom, and were obtained by dividing the squared difference in a (1-1) comparison by 2, in a (twice 1-2) comparison by 6, and in a (2-2) comparison by 4 (7, pp. 291-295).

Table 4 shows an analysis of covariance for the 842 independent intralot comparisons. The average regression equation, based on the pooled intralot variances and covariances and the general means, was  $V=801.7-240.5R$ . This straight line is plotted in figure 4.

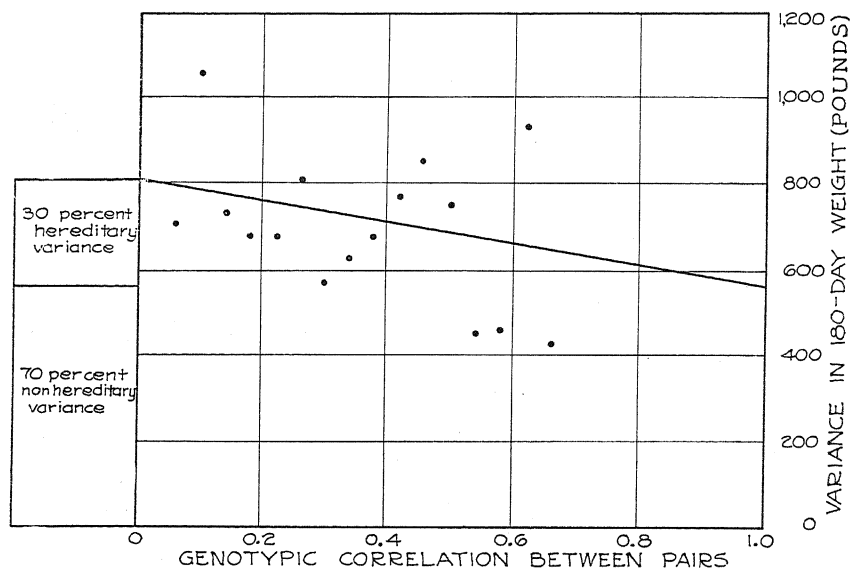


FIGURE 4.—Regression of the variance in 180-day weights on the relationship between members of 842 pairs of pigs.

The standard error of this regression coefficient ( $-240.5$ ) is approximately 33. For each 1 percent increase in relationship there was an average decrease of 2.4 in the variance between pairs.

From this regression equation,  $V=801.7$  when  $R=0$ . This would be the variance expected between unrelated pigs which were lot mates and would include all the variance due to their differences in heredity plus all the variance due to environmental variations, known and unknown, within the lots in these experiments. Substituting  $R=1.0$ , yields  $V=561.2$  as the variance expected within an isogenic line kept under the same conditions as these pigs were kept. Hence,  $\frac{801.7-561.2}{801.7}=0.30$ =the fraction of the original variance which was due to differences in heredity.

Because most of the relationships on which this regression line was fitted were low, and because the line curves downward as  $R$  increases if dominance exists and if there are epistatic combination effects among the genes affecting 180-day weights, this method overestimates the variance which would remain in an isogenic line and hence underestimates heritability. No basis for estimating the magnitude of this discrepancy is apparent. All that can be said concerning it is that this estimate of 30 percent includes all of the additively genetic part of the variance plus an uncertain but probably small fraction of that due to dominance and epistasis.

TABLE 4.—Regression of variance in 180-day weight ( $V$ ) on genetic relationship ( $R$ ) among lot mates, maternal sibs excluded

Source of variance	Degrees of freedom	Sum of squares for relationship ( $R$ ) <sup>1</sup>	Sum of cross products ( $RV$ )	Sum of squares for variance ( $V$ ) <sup>2</sup>	Regression coefficient
Between lots.....	86	1.91	+1,382	209,231,269	
Within lots.....	756	10.80	-2,598	634,651,075	-240.5
Total.....	842	12.71	-1,216	843,882,344	-95.6

<sup>1</sup> Mean  $R=0.340$ .

<sup>2</sup> Mean  $V=719.9$ .

Each relationship between genotypes is a statement of the degree of likeness which would most probably result from the normal operation of the Mendelian laws of inheritance and is not an actually measured observation. For example, the computed relationship between pigs 145 and 185 was 0.206 but if the actual identity of all their genes could be known, each weighted by how much it affects 180-day weight, it could readily happen that 0.18 would be the real measure of likeness, while in another pair with the same coefficient the real likeness might be 0.23. This introduces some random error into the independent variable and thus tends to blur or flatten the regression and to make the estimate of heritability too low. This would be important if only a few genes were involved, as then the Mendelian sampling error would be large relative to the range. But it would be unimportant if, as seems more probable, a very large number of genes affect 180-day weight which, after all, is a complex result of a large number of body parts and physiological processes many of which in turn are themselves genetically complex.

It should also be remembered that the environmental variance here is that within lots. In a population from many lots and many years there would almost certainly be a larger environmental variance and correspondingly lower heritability due to environmental differences between lots and between years.

#### INTRASIRE REGRESSION OF OFFSPRING ON DAM

From the mates and offspring of 15 boars which each sired pigs out of 2 or more sows, an intrasire correlation of 0.15 between the weight of the offspring and the weight of the dam was observed. This analysis included 756 pigs from 150 matings. The dams were themselves selected partly on their own 180-day weight and were much less variable than their offspring, as figure 5 shows. The intrasire

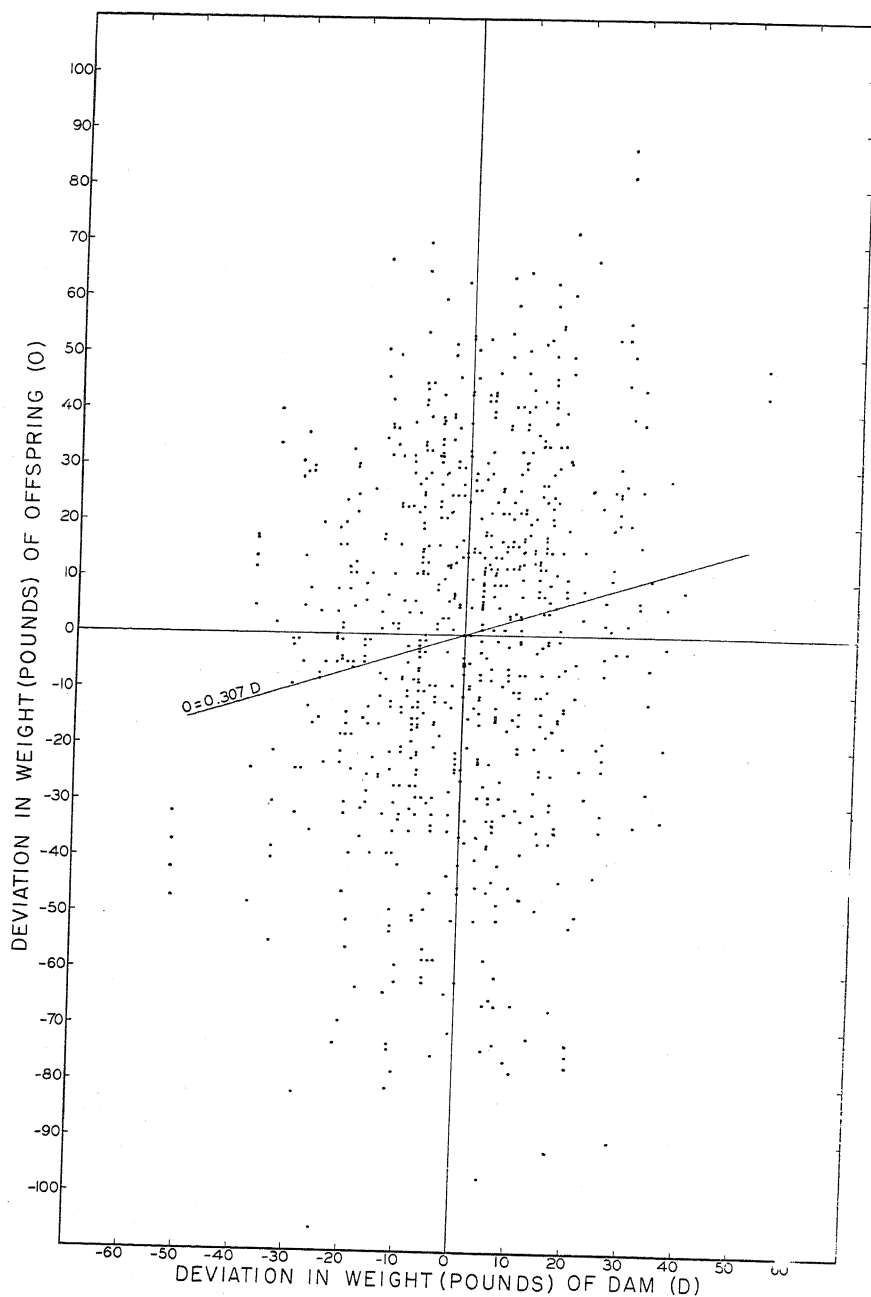


FIGURE 5.—The intrasire regression of the 180-day weight of the offspring on the 180-day weight of the dam; points plotted as deviations from the average of offspring and from the average of mates of each sire.



standard deviation of the offspring was 32.5 pounds, while that of the dams was 16.0 pounds. This smaller variation in the dams' weights makes it necessary to use regression of offspring (the unselected variable) on dam (the selected variable), rather than the correlation between them (9), as a basis for estimating heritability. The intrasire regression coefficient of the weight of the offspring on the weight of the dam was 0.31, which is to say that a difference of 1 pound between the 180-day weights of 2 sows mated to the same boar on the average resulted in 0.31 of a pound difference in the 180-day weight of their offspring. Doubling this (because only half of the offspring inheritance comes from the dam) yields 0.62 as the heritability of differences in 180-day weights among gilts which later were mated to the same sire. This fraction includes all of the additively genetic variance plus less than half of the epistatic variance.

The regression of the offspring weight on the dam's weight is plotted in figure 5, as are the individual deviations from this line. The weight of each offspring is expressed as a deviation from the average of all offspring of that sire and the weight of the dam is expressed as a deviation from the average of all dams mated to that sire. This eliminates the differences between the means of mates and means of offspring of different sires and makes the dam-offspring pairs from the different sires visually comparable.

#### CORRELATIONS BETWEEN SIBS

Correlations between the 180-day weights of litter mates and of paternal half-sibs were deduced from the analysis of variance (3) within litters and between litters from the same sire. Differences between farrowing seasons were eliminated by analyzing each of the eight farrowing seasons separately and then combining the results into an average intraseason analysis which is shown in table 5.

TABLE 5.—Intraseason analysis of variance of 180-day weight due to litter and sire

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between sires	29	116,991	4,034.2
Between litters by the same sire	230	495,284	2,153.4
Between litters	259	612,275	2,364.0
Within litters	1,127	740,344	656.9
Total	1,386	1,352,619	975.9

The variance in table 5 was separated as follows on the basis of sources of differences between individual pigs:

$A$  = variance within litters (= 656.9)

$B$  = additional variance between paternal half-sibs

$C$  = additional variance between nonsibs

Consequently,  $A+B+C$  is the variance found within random pairs of nonsibs,  $A+B$  is the variance found within random pairs of paternal half-sibs, and  $A$  is the variance found between litter mates.

The mean square between litters by the same sire (2,135.4) contains

$A$  plus  $\left(\frac{1,394}{267} - \frac{4.73}{1,394}\right)B$ .<sup>5</sup> Then  $B=286.8$ .

<sup>5</sup> The figures in parentheses are the average number of pigs per litter minus the variance of litter size divided by the total number of pigs. Generally the last term is negligibly small and may be dropped without serious error. For more detailed information on this correction see Winsor and Clarke (8).

The mean square between sires (4,034.2) contains the mean square between litters by the same sire plus  $\left(\frac{1,394}{37} - \frac{303.2}{1,394}\right)C$ . Then  $C=50.2$ .

In percentages of  $A+B+C$  the variance ascribable to each of the three sources is:

$$A=656.9=66.1 \text{ percent of total}$$

$$B=286.8=28.9 \text{ percent of total}$$

$$C=50.2=5.0 \text{ percent of total}$$

The variance within litters was 28.9 plus 5.0 or 33.9 percent less than the total variance which would have existed in a population of pigs all from different litters and each by a different sire. This corresponds to an intraseason correlation of 0.339 for 180-day weights of litter mates.

Paternal half-sibs are alike only in that they have the same sire. The correlation between paternal half-sibs corresponds to the fraction which the  $C$  term constitutes of  $A+B+C$ . This was 0.051. If all the pigs had been by the same sire but out of different dams, the total variance would have been reduced 5.1 percent. Since these correlations were deduced from mean squares which differed with high statistical significance, these correlations were likewise highly significant.

The 267 litters included in this study were from 151 sows. The presence of sets of 2 or more litters from the same sow made possible the study of the correlation between maternal half-sibs. Because a sow could produce only 1 litter in 1 farrowing season, maternal half-sibs of necessity must have been born in different years or at least in different seasons of the same year; hence, it was not possible to eliminate the effect of year and season in computing the correlation between them.

TABLE 6.—Analysis of variance of 180-day weight showing the effect of dam

Source of variance	Degrees of freedom	Sum of squares	Mean square
Between half-sib litters from the same dam	94	228,490	2,430.8
Between full-sib litters from the same dam	22	31,385	1,426.6
Between litters from the same dam	116	259,875	2,240.3
Between dams	150	543,739	3,624.9
Between litters	266	803,614	3,021.1
Within litters	1,127	740,344	656.9
Total	1,393	1,543,958	1,108.4

In table 6 the 266 degrees of freedom between litters were broken down into 150 between dams and 116 between litters from the same dam. The mean square between litters from the same dam included some full-sib litters as well as some litters that were only half-sibs. A separate analysis was made of the 42 full-sib litters. The degrees of freedom, sum of squares, and mean squares from this analysis are included in table 6. By their use the mean square between litters from the same dam was broken down into that between half-sib litters from the same dam and between full-sib litters from the same dam.

The variance in table 6 comes from four different sources:

$A$  = Variance within litters =  $656.9 = 66.8$  percent

$J$  = Additional variance for full sibs born in different litters =  $127.4 = 13.0$  percent

$M$  = Additional variance for maternal half-sibs =  $66.8 = 7.0$  percent

$Y$  = Additional variance between nonsibs =  $129.8 = 13.2$  percent

When the dams were the same and the sires were different the variance in 180-day weight was reduced 13.2 percent ( $Y$ ). This corresponds to a correlation of 0.132 between maternal half-sibs, and might be expected to consist of a genetic fraction like that between paternal half-sibs (0.051) and an environmental fraction due to permanent differences in the ability of the dams to be good mothers and nurses. Subtracting 0.051 from 0.132 yields 0.081 as an estimate of the importance of these differences between dams, which in part may have been genetic characteristics of the dams but certainly were environmental as far as each pig's own 180-day weight was concerned.  $M+Y$  gives 0.202 as the correlation between full sibs not litter mates.  $M$  or 0.070 would include some dominance and some epistatic effects, along with the genetic effect of having the same sire. Using 0.051 for the latter yields only 0.019 for the increase which dominance and epistasis make in the full-sib correlation over the half-sib correlation. This indicates that they were not very important in these data, but this conclusion has a high sampling error because of the limited numbers involved, the selection practiced among parents, and because the estimate is based on differences between mean squares, each subject to a sampling error.

The correlation between litter mates is  $J+M+Y=0.332$ , which is almost the same as the 0.339 observed directly. Thirteen percent ( $J$ ) of the total variance in 180-day weight was caused by temporary influences which affected litter mates alike but full-sib litters from the same dam differently. Much of this was probably caused by differences in age of dam, yearly and seasonal changes, incidence of infections, temporary state of health of the dam, and other external factors that affected litter mates alike. This 13 percent is clearly an environmental part of the correlation between litter mates, but some of  $Y$  may also be environmental.

In summary, the following correlations between the 180-day weights of certain sibs were observed:

Litter mates (intraseason) .....	0.339
Full sibs not litter mates .....	.202
Maternal half-sibs .....	.132
Paternal half-sibs (intraseason) .....	.051

The correlation between paternal half-sibs in a random breeding population is composed of about one-fourth of the additive genetic fraction of the variance plus a small part (probably less than one-sixteenth) of the variance due to epistatic interactions of nonallelic genes plus the environmental fraction of the variance multiplied by the correlation between the environments of paternal half-sibs (11). Since the paternal half-sib correlation was computed on an intraseason basis and there was no attempt to treat the progeny of one sire differently from the progeny of another sire, the environmental correlation between paternal half-sibs should be close to 0. A comparison of the correlation between full sibs not litter mates (0.202) with the maternal and paternal half-sib correlations (0.132 and 0.051) indicates that the epistatic fraction of the variance in these data is small.

This, coupled with the fact that only a small part (one-sixteenth or less) of this variance is included in the paternal half-sib correlation, indicates that the epistatic part of the variance in the correlation may be disregarded without introducing much error. On the basis of these assumptions the correlation between paternal half-sibs (0.051) is multiplied by 4 to give 0.204 as the additive genetic fraction of the variance. This estimate is probably a little below the true value since the sires were to some extent themselves selected because of their own 180-day weights and this would tend to make the correlation between paternal half-sibs a little lower than would be found in a population of pigs from truly unselected parents. It should also be remembered that whatever sampling error was present in the paternal half-sib correlation was multiplied by 4 in getting the figure 0.204.

#### DISCUSSION

The degree of heritability of a characteristic is a measure of the amount of the observed variance that can be attributed to the additive effects of genes. All methods of estimating heritability depend in some manner on the degree to which related animals resemble each other more than unrelated ones do. The different kinds of genetic relationships for estimating heritability include various amounts of the variance due to dominance deviations, epistatic interactions, or environmental similarities or differences. It is not surprising, therefore, that estimates of heritability based on different kinds of relationships should not agree exactly, because some relationships are less affected than others by environmental variations, and by epistatic or dominance effects of genes.

The different estimates of heritability of 180-day weight derived from this study are summarized in table 7. This table also includes

TABLE 7.—Summary of hereditary variance: estimates from different sources

Percent of hereditary variance	How derived	Remarks
(a) 20.4.....	4 times paternal half-sib correlation.	Very little dominance, epistatic, or environmental correlations present in this figure. Large sampling error because of the small number of sires. Selection of sires probably makes this figure a little low.
(b) 30.4.....	Twice the intrasire offspring-dam correlation.	Probably too low because of the reduced variability of the dams resulting from selection. Sampling error small. Includes slightly more epistatic variance than (a).
(c) 40.4.....	Twice the correlation between full sibs not litter mates.	Subject to large sampling error because of small amount of data. Dominance and permanent effects of dam's nursing ability would make this figure too high, but year-to-year differences would tend to make it too low.
(d) 62.0.....	Twice the intrasire regression of offspring on dam.	More reliable than (b) because the reduced variability of the independent variable (dam) has no effect on the regression coefficient.
(e) 30.0.....	Regression of variance on genetic relationship.	Includes at least a little dominance and epistatic variance, but less environmental variance than above methods since it is a fraction of differences between pigs in the same lot.

a brief description of the method of deriving each estimate and remarks on its reliability. From a study of this table it would appear that at least 30 and perhaps more than 40 percent of the individual variance in 180-day weight was due to hereditary differences in these pigs.



## SUMMARY AND CONCLUSIONS

Data were analyzed to determine the influence of hereditary and certain environmental factors on the 180-day weights of 1,394 Poland China pigs in 267 litters which were out of 151 sows and by 23 boars. The average weight of the pigs was 180.3 pounds and the intraseason standard deviation was 31.3 pounds.

A correlation of  $-0.17 \pm 0.03$  between weight of pig and percent of inbreeding and a regression coefficient indicating an average decrease of 0.76 of a pound in weight for each 1 percent increase in inbreeding were observed. The multiple correlation of 180-day weight as dependent on weaning weight and birth weight was 0.58. From the multiple regression equation it was concluded that weaning weight was twice as important as birth weight in predicting 180-day weight. Seasonal differences, year-to-year changes in environment, and differences in age of dam influenced the weight at 180 days, although the effect of the dam's age was small. Gilts weighed about 4 percent less than barrows and boars at 180 days.

The intralot regression of the variance in 180-day weight on the genetic relationship between 843 pairs of pigs indicated that about 30 percent of the intralot individual variance was due to genetic factors. The intrasire regression of offspring weight on dam's weight (0.31) gave an estimate of 62 percent for the hereditary part of the variance in individual weight of gilts which later were mated to the same boar.

A correlation of 0.051 was observed between paternal half-sibs, 0.132 between maternal half-sibs, 0.152 between dam and offspring, 0.339 between litter mates, and 0.202 between full sibs not litter mates. Because the parents were rather highly selected groups, these correlations very likely were underestimates of what would occur in an entirely unselected population. The paternal half-sib correlation gave a figure of 20 percent, the correlation between full sibs not litter mates gave a figure of 40 percent, and the offspring-dam correlation gave a figure of 30 percent for the part of variance in 180-day weight due to the action of genetic factors combining additively.

The general conclusion was drawn that at least 30 percent and perhaps more than 40 percent of the individual variance in 180-day weight was due to the additive effects of genes.

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## CONTENTS

	Page
The Effects of Leafhopper Feeding Injury on Apparent Photosynthesis and Transpiration of Apple Leaves (Key No. Ind.-30) G. EDWARD MARSHALL, N. F. CHILDERS, and HOWARD W. BRODY	265
Effect of Erosion on Fertility Changes in the Shelby Loam Profile (Key No. S-3) D. M. WHITE and C. L. W. SWANSON	283
Factors Affecting Distribution and Severity of Black Root Rot of Apple Trees (Key No. G-1265) J. S. COOLEY	299



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# JOURNAL OF AGRICULTURAL RESEARCH

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No. 6

## THE EFFECTS OF LEAFHOPPER FEEDING INJURY ON APPARENT PHOTOSYNTHESIS AND TRANSPIRATION OF APPLE LEAVES<sup>1</sup>

By G. EDWARD MARSHALL, *associate in entomology, Purdue University Agricultural Experiment Station*; N. F. CHILDERS, *associate in horticulture*, and HOWARD W. BRODY, *research assistant in horticulture, Ohio Agricultural Experiment Station*<sup>2</sup>

### INTRODUCTION

The leafhoppers that attack apples have received relatively little attention from investigators and growers, probably because they do not cause immediate injury to the fruit itself as do codling moths, curculios, and similar chewing insects, and probably, too, because their greatest injury to the leaves appears late in the season when the fruit is almost mature.

Two types of leafhopper injury occur on apple leaves: (1) A yellowish-green stippling on the upper leaf surface caused by species of *Typhlocyba*, *Erythroneura*, and some *Empoasca*—the mesophyll feeders (1, 4, 9, 10, 11)<sup>3</sup>; and (2) a yellowing, puckering, and wilting of the leaf caused by one or more species of *Empoasca*—the vein feeders (11, 12, 13).<sup>4</sup> There appear to be no published data which show the effects of vein- or mesophyll-feeding leafhoppers on the metabolic processes of apple leaves. The literature deals largely with descriptions of visible leaf injury (3, 6, 8).

It is the purpose in this paper to point out the effect of various degrees of leafhopper feeding injury on apparent photosynthesis and transpiration of apple leaves. Seasonal trends in leafhopper population and the effect of feeding injury on leaf anatomy also are discussed.

### MATERIALS AND METHODS

The leafhoppers used in this study were collected from a large McIntosh apple tree growing in an experimental orchard in southern Indiana and were shipped to Ohio State University by special delivery in hardware-cloth cages. The time in transit was less than 24 hours. The cages were ellipse-shaped, about 10 inches in height by 4 inches in diameter, and were filled with damp excelsior and wrapped with cheesecloth. This technique was employed in all seasons of the year; fresh material was used in the cages in summer. The population counts were made at 8 evenly distributed locations on the tree (fig. 1) during the summer of 1940. One thousand leaves were inspected weekly for the tree as a whole, 5 groups of 25 leaves each at each of the 8 locations.

When the leafhoppers arrived at the laboratory, they were released into special cages which covered the shoots of potted Stayman Winesap

<sup>1</sup> Received for publication October 8, 1941.

<sup>2</sup> The authors are indebted to the Tobacco By-Products & Chemical Corporation, Louisville, Ky., for a fellowship in connection with this study. Helpful advice from Prof. J. J. Davis, head, Department of Entomology, Purdue University Agricultural Experiment Station, is gratefully acknowledged.

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 280.

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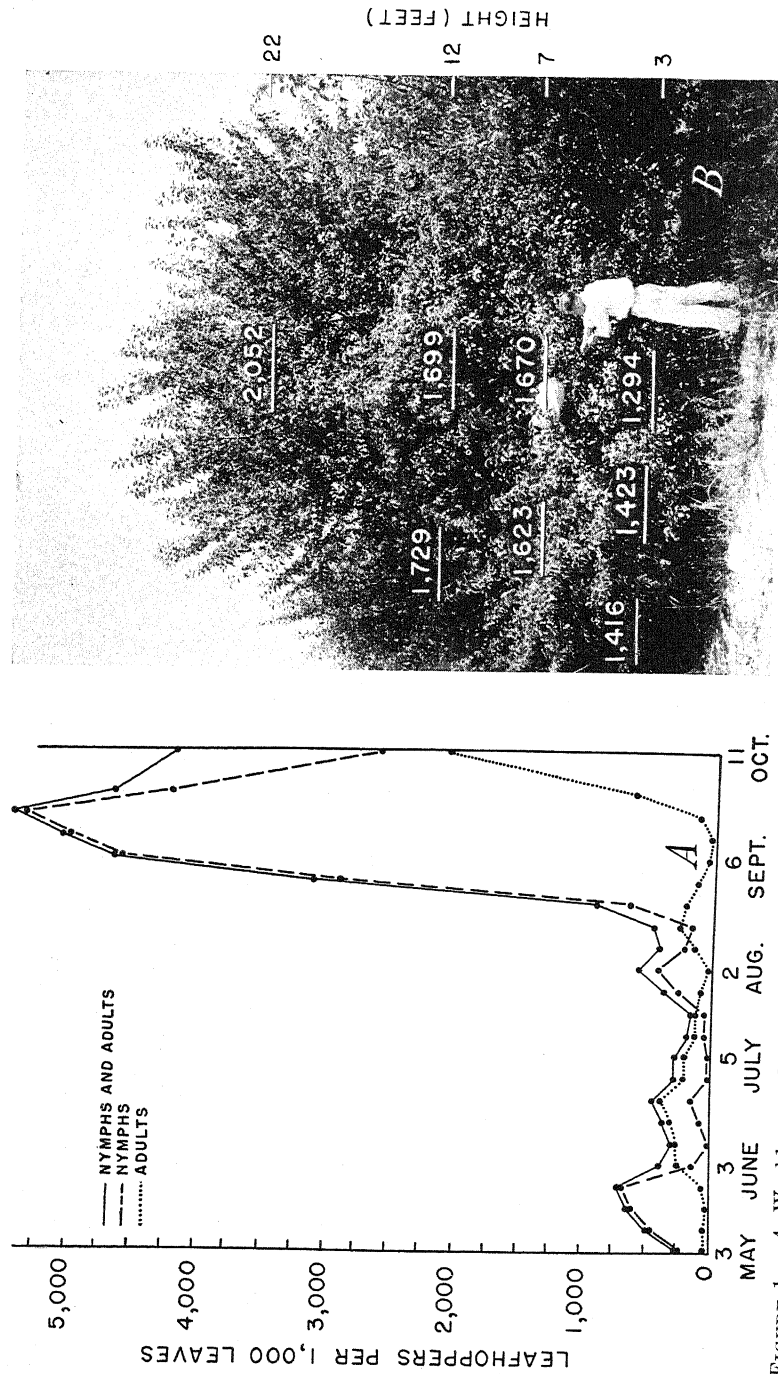


Figure 1-4. Weekly population trend of leafhoppers on a McIntosh apple tree in southern Indiana during the 1940 season; B, the tree from which the leafhoppers were collected. The figures superimposed on the photograph are average numbers of insects per 1,000 leaves found each week in counts at 8 distributed locations at 3-, 7-, 12-, and 22-foot levels.

apple trees. The cages were made of  $\frac{1}{4}$ -inch-mesh hardware cloth covered with cheesecloth, and had small cellulose acetate windows through which the insects could be observed.

Photosynthesis and transpiration measurements were made at Ohio State University by the method of Heinicke and Hoffman (7), both outdoors and in the environment-control chamber (2). The metabolic activity of 12 leaves was studied in each experiment; 6 of the leaves were treated and 6 remained as checks. After a ratio between the rates of photosynthesis and transpiration of the 2 groups of leaves had been determined, a sleeve cage of insects was placed over one of the shoots as shown in figure 2. The insects were permitted to feed on the foliage for a given period, after which they were removed and the metabolism studies were resumed. Any change in the ratio after the feeding period as compared with the ratio before feeding was interpreted as the result of insect injury. The insects were permitted to feed at several successive periods in a given experiment.

Apple leaves upon which leafhoppers had been permitted to feed were examined microscopically, in cross section. The leaf material was mounted in paraffin, cut on a rotary microtome, and stained with Heidenhain's iron-alum haematoxylin.

Analysis of variance and significance of data were determined according to Fisher's tables for  $F$  and  $t$ .<sup>5</sup>

## EXPERIMENTAL RESULTS

### POPULATION STUDIES

Counts of leafhoppers in the experimental orchard were made each week, beginning May 3, 1940. After the overwintering population of *Erythroneura* species had died about the middle of May, *Typhlocyba pomaria* McA., the white apple leafhopper, was the only species present in significant numbers until late in the summer. The graph in figure 1 shows the weekly population of all leafhoppers, adults and nymphs, on the mature McIntosh tree. The first peak in the curve represents almost entirely the species *T. pomaria*; the second, several species of *Erythroneura*; the third, *T. pomaria* and some *Erythroneura*; and the last, *T. pomaria* and various species of *Erythroneura* in the ratio of 2.83 *T. pomaria* to 1 of *Erythroneura*. The figures on the photograph in figure 1, B, show the average number of leafhoppers per 1,000 leaves found each week in counts at the respective locations up to August 4, 1940. It will be noted that the insects were more abundant near the top of the tree.

Eggs of *Typhlocyba pomaria* were laid in the bark of 1- to 4-year wood in the fall and these overwintered before hatching; *Erythroneura* overwintered in the adult stage under leaves and other protective plant debris. Population counts of *Erythroneura* during the winter of 1939-40 revealed an average of 160 adults per square yard in fallen leaves and grass in the orchard. The leafhoppers were more numerous near the drip of the branches where ground cover was heavier.

### EFFECTS ON PHOTOSYNTHESIS AND TRANSPIRATION OF APPLE LEAVES CAUSED BY FEEDING OF APPLE LEAFHOPPERS

One hundred leafhoppers per leaf were released in a sleeve cage over apple shoots after the pretreatment ratio for photosynthesis and transpiration had been established over a 3-day period. The insects were

<sup>5</sup> The statistical analyses were carried out under the direction of Mrs. Z. E. Alberts, Department of Zoology and Entomology, Ohio State University.

permitted to feed for 3 days, after which leaf activity was measured again. The data in table 1 and figure 3, *A*, show that the rate of photosynthesis was reduced 25 percent and transpiration 26.7 percent by the feeding of leafhoppers at the rate of 100 per leaf for 3 days, or 7,200 "leafhopper hours" per leaf (see fig. 4, *A, a*, for apparent leaf injury).

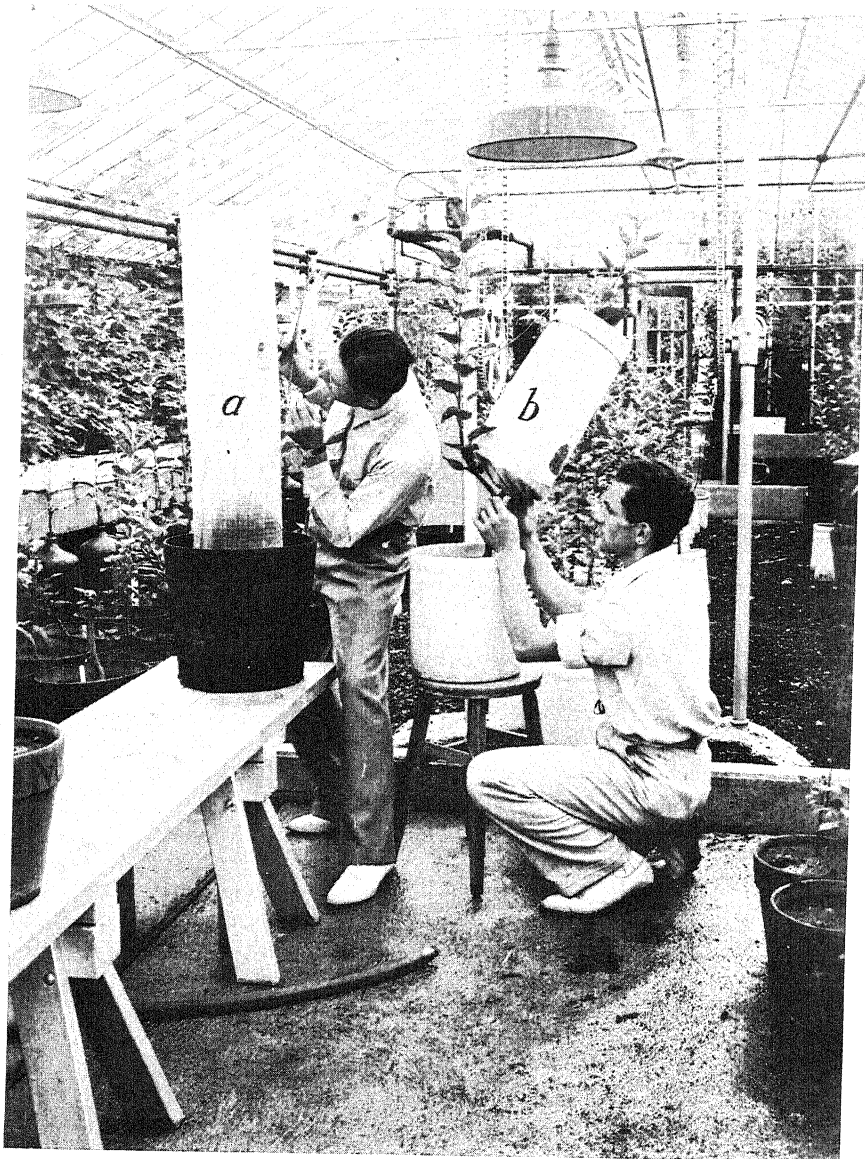


FIGURE 2.—Experimental trees with two shoots were grown in 5-gallon stone crocks; one of the shoots was used in the feeding experiment, the other served as a check. A definite number of insects per leaf were transferred from the stock cage (*a*) to the test cage (*b*) for each successive feeding period between photosynthesis-transpiration determinations.



TABLE 1.—The effect of apple leafhopper injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment A

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)		Test/Check ×100	Expected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)		Test/Check ×100	Expected rate <sup>2</sup>
		Check	Test				Check	Test		
	Milli-grams	Milli-grams	Milli-grams		Per-cent <sup>2</sup>	Gram	Grams	Grams		Per-cent
Mar. 3	19.9	10.5	14.0	102.9		0.302	0.56	0.83	148.2	
Mar. 4	19.0	7.0	7.8	97.1		.305	.94	1.18	125.5	
Mar. 5	18.0	17.5	20.8	118.3		.292	1.10	1.50	136.4	
Average				106.1					136.7	
Mar. 9	19.2	15.1	15.9	105.3	99.3	.251	1.47	1.55	105.4	77.1
Mar. 10	17.4	13.9	9.1	65.5	61.8	.267	1.62	1.48	91.3	66.8
Mar. 11	17.7	12.9	11.7	90.7	85.5	.230	1.63	1.65	101.9	74.6
Mar. 12	17.1	13.1	7.5	57.3	54.0	.261	1.56	1.63	104.5	76.5
Average				79.7	75.1				100.7	73.8
Mar. 16				81.8	77.1	.255	1.58	1.77	112.0	82.0
Mar. 17	17.7	20.3	16.6			.257	1.60	1.62	101.3	74.1
Mar. 18				82.8	78.1	.249	1.62	1.68	103.7	75.9
Mar. 19	16.8	18.6	15.4			.231	1.97	1.43	72.6	53.1
Average				82.3	77.6				97.4	71.3
Apr. 1	17.3	12.3	10.0	81.3	76.6	.264	1.75	1.75	100.0	73.2
Apr. 2						.291	2.06	1.65	80.1	58.6
Apr. 3	18.2	14.1	11.9	84.4	79.6	.249	2.13	1.76	82.6	60.4
Apr. 4	16.5	15.0	13.9	92.7	87.4	.255	2.10	1.25	59.5	43.5
Apr. 5	18.2	15.8	11.3	71.1	67.0	.265	1.44	1.56	108.3	79.3
Apr. 6	18.3	15.8	12.7	80.4	75.8	.250	1.97	1.41	71.6	52.5
Average				82.0	77.3				83.7	61.2
Apr. 12	17.8	17.1	12.8	74.9	70.6	.262	1.80	1.77	98.3	71.9
Apr. 13	17.4	14.1	10.6	75.2	70.9	.257	1.83	1.82	99.5	72.8
Apr. 14	17.7	14.2	11.7	82.4	77.7	.253	1.83	1.54	84.2	61.6
Apr. 15	19.4	16.3	16.7	102.5	97.6	.261	1.47	1.20	81.6	59.7
Apr. 16	18.0	17.5	17.3	98.9	93.3	.254	1.13	.96	85.0	62.2
Apr. 19	17.7	14.0	9.9	70.9	66.9					
Average				84.3	74.3				89.7	65.6
Apr. 26	15.9	12.3	11.0	89.4	84.3	.250	1.64	1.55	94.5	69.1
Apr. 28						.256	1.83	1.33	72.1	52.7
Apr. 29	16.8	15.5	6.3	40.6	38.3	.248	1.86	1.64	88.2	64.5
Apr. 30	16.5	15.1	8.8	58.3	55.0	.251	1.92	1.53	79.7	58.3
May 1	17.3	12.0	7.3	60.8	57.3	.250	1.90	1.35	71.1	52.0
May 2	17.7	14.5	6.6	45.5	42.9	.228	2.32	1.78	76.7	56.1
Average				58.9	55.8				80.4	59.6
May 21	16.5	4.4	.9	20.5	19.3	.216	1.32	.93	70.5	51.5
May 22	15.6	6.3	0	0	0	.269	1.18	.76	64.4	47.0
May 23	16.3	8.3	3.6	43.4	40.9	.275	1.14	.75	65.8	48.2
Average				21.3	20.1				66.9	48.9

ANALYSIS OF VARIANCE OF  $\frac{\text{TEST}}{\text{CHECK}} \times 100$ 

Item	Degrees of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis	6	14, 158.62	2, 359.8	3.27	Significant.
	21	15, 174.66	722.6		
Transpiration	6	9, 553.04	1, 592.2	10.54	Highly significant.
	24	3, 626.39	151.1		

<sup>1</sup> Between each series of measurements the leafhoppers were permitted to feed.<sup>2</sup> Test after treatment $\frac{\text{Check after treatment}}{\text{Test before treatment}} \times 100 = \text{Percent expected rate}$ 

Check before treatment

From these data it could be inferred that 300 leafhoppers per leaf (assuming a constant area of about 50 cm.<sup>2</sup> for all leaves) may cause a reduction in photosynthesis and transpiration of about 1 percent in 1 hour.

Further injury (fig. 4, A, b) on the same leaves did not cause a significant reduction in either process. It was not until 4 weeks of

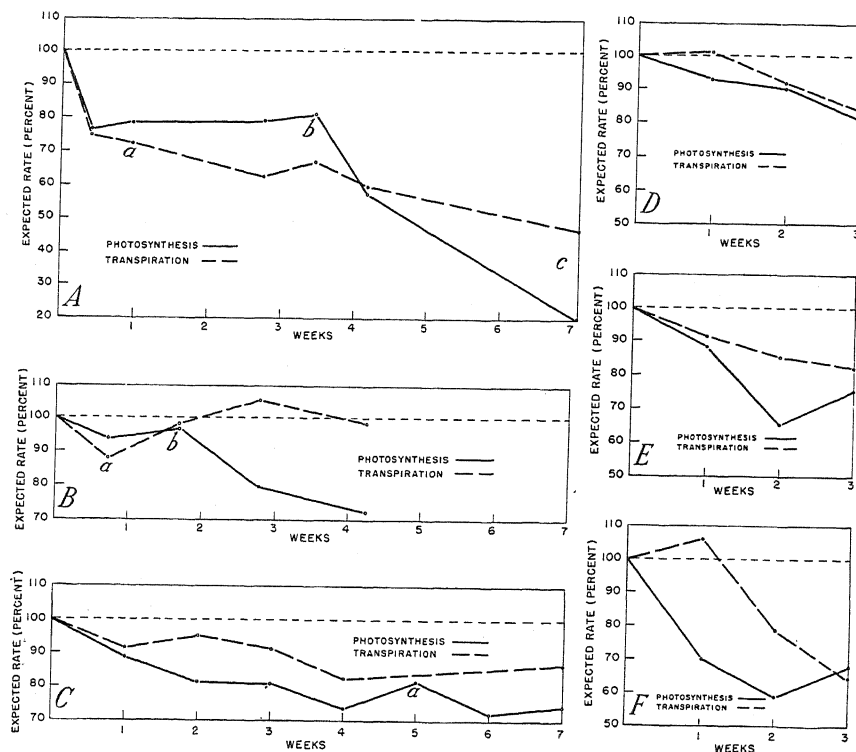


FIGURE 3.—A, B, C, D, and E, based on data from experiments of similar designation, show the effects of mesophyll-feeding by apple leafhoppers on apparent photosynthesis and transpiration of Stayman Winesap apple leaves. F shows the effect of the vein-feeding potato leafhopper, *Empoasca fabae*, on apple leaf metabolism. The value 100 represents photosynthesis and transpiration of the check leaves for every determination.

feeding with a slightly reduced insect population that further measurable reductions occurred. Seven weeks of feeding reduced the rate of photosynthesis 80 percent and transpiration 56.2 percent. At this time the leaves appeared severely injured; the surfaces were yellowish green, the spots had coalesced over the entire area, and there was a slight curling and burning at the margins (fig. 4, A, c). The

reduction in photosynthesis was significant and in transpiration highly significant.

#### EXPERIMENT B

In similar experiments but with a smaller population of insects (50 to 60 per leaf), there was a reduction in photosynthesis but the

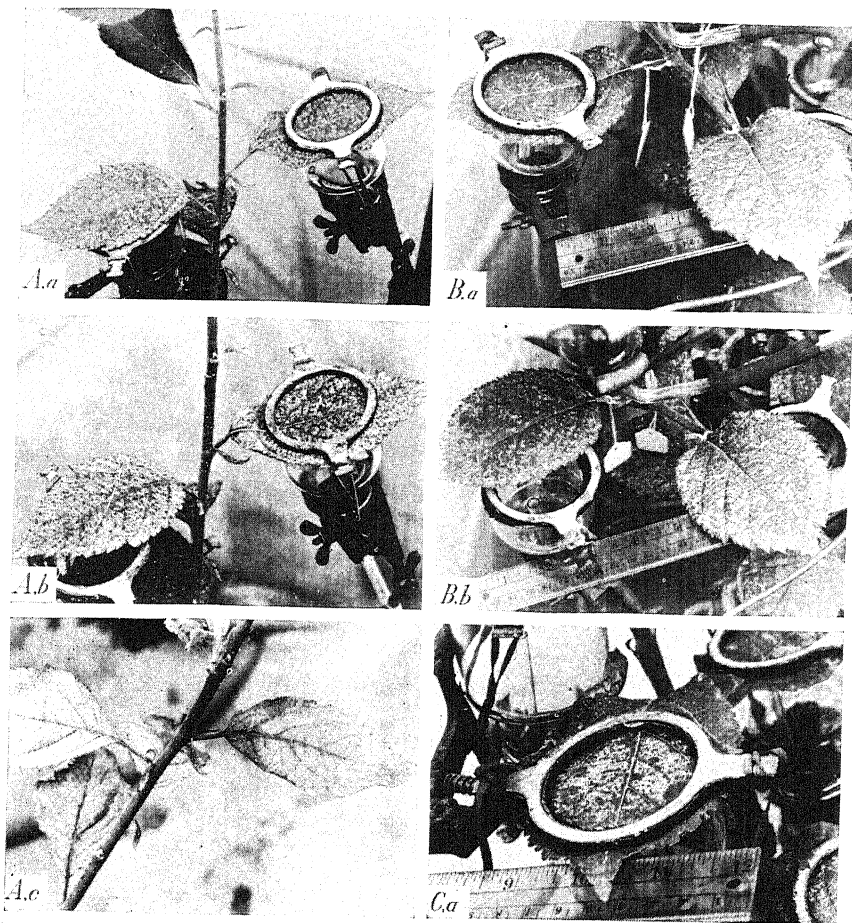


FIGURE 4.—Stages of feeding injury at positions marked on the photosynthesis-transpiration curves in figure 3; for example, *A, a*, shows apparent feeding injury when photosynthesis was reduced approximately 25 percent and transpiration 27 percent as indicated at point *a* in figure 3, *A*.

rate of transpiration was not significantly affected (table 2, fig. 3, *B*). Four weeks of feeding was accompanied by a significant reduction in photosynthesis of 26.4 percent. (See fig. 4, *B, a*, and *B, b*, for apparent injury at stages specified in fig. 3, *B*.)

TABLE 2.—The effects of apple leafhopper injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment B

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)		Test Check × 100	Expected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)		Test Check × 100	Expected rate <sup>2</sup>
		Check	Test				Check	Test		
	Milli-grams	Milli-grams	Milli-grams		Per-cent <sup>2</sup>	Gram	Grams	Grams		Per-cent
Mar. 16	19.4	19.4	19.3	99.5						
Mar. 17	16.4	22.1	22.2	100.5		0.248	1.78	1.46	82.0	
Mar. 18	16.4	16.5	15.5	93.9		.240	1.81	1.69	93.4	
Mar. 19	17.6	18.3	17.9	97.8		.237	1.87	1.66	88.8	
Average				97.9					88.1	
Mar. 26	18.0	19.7	20.1	102.0	104.0	.244	1.33	1.06	80.0	90.8
Mar. 27	20.7	29.0	28.1	97.0	99.1	.252	1.31	.92	70.2	80.0
Mar. 28	22.4	24.6	18.0	73.2	74.7	.269	1.47	1.48	100.7	114.3
Mar. 29	17.7	16.9	17.6	104.1	106.3	.260	1.69	1.25	74.0	84.0
Mar. 30	17.2	17.0	13.7	80.6	82.3	.279	1.59	1.38	86.8	98.5
Mar. 31	16.9	12.2	12.7	104.1	106.3	.251	1.12	.84	75.3	85.5
Average				93.5	95.5				81.2	92.2
Apr. 7	18.6	15.1	14.4	95.3	97.4	.258	1.44	1.33	92.4	105.0
Apr. 8	17.0	14.6	15.7	107.5	109.8	.241	1.85	1.74	94.1	106.8
Apr. 9	16.8	15.3	13.4	87.8	89.7	.260	1.58	1.55	98.1	111.4
Apr. 10	18.2	10.4	10.1	97.1	99.2	.246	1.22	1.02	83.6	94.9
Apr. 11						.259	1.46	1.27	87.0	98.8
Average				96.9	99.8				91.0	103.4
Apr. 20	30.6	23.5	16.7	71.1	72.6	.250	.86	.88	102.3	116.1
Apr. 21	25.6	22.2	18.5	83.3	85.1	.256	1.21	1.26	104.1	118.2
Apr. 22	17.5	14.7	11.9	81.0	82.7	.252	1.39	1.29	92.8	105.3
Apr. 23	17.9	13.4	9.5	70.9	72.4	.253	1.61	1.52	94.4	107.2
Apr. 24	15.9	14.7	11.6	78.9	80.6	.248	1.47	1.55	105.4	119.6
Apr. 25	18.1	24.0	21.1	87.9	89.8	.262	1.63	1.48	90.8	103.1
Average				78.9	80.5				98.3	111.6
May 5	16.3	14.3	10.0	69.9	71.4	.259	1.66	1.61	92.0	110.1
May 6	15.8	11.2	10.6	94.6	96.6	.259	1.98	1.59	80.3	91.1
May 7	17.7	14.1	7.3	51.8	52.9	.277	1.81	1.74	96.1	109.1
Average				72.1	73.6				92.5	103.4

ANALYSIS OF VARIANCE OF  $\frac{\text{TEST}}{\text{CHECK}} \times 100$ 

Item	Degrees of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis	4	2153.45	538.36	4.28	Significant.
	18	2263.59	125.76		
Transpiration	4	901.98	225.5	3.40	Significant variance but not reduction.
	18	1197.42	66.37		

<sup>1</sup> Between each series of measurements the leafhoppers were permitted to feed.<sup>2</sup> See footnote 2, table 1, for method of calculation.



## EXPERIMENTS C AND D

Experiments were run on experimental leaves in the laboratory to duplicate the population of insects in the field. At the time weekly population counts were made in the field, leafhoppers (adults and nymphs) were collected and shipped from the Indiana orchard to the environment-control chamber. The concentration of insects, species ratio, and interval between changes in insect population on the test leaves were adjusted to make the duration of the experiment in the laboratory shorter than an entire summer season. The data can be interpreted, however, as typical of the results of a season of leafhopper activity.

The technique employed in handling the leafhoppers in these experiments differed from that in the previous experiments. The leafhoppers were released in the leaf cups (7) on the lower surfaces of the apple leaves instead of in cheesecloth cages. According to Wigglesworth (14, p. 198), insects respire at the rate of approximately 3 mg. of carbon dioxide per gram of insect weight per hour. A leafhopper, then, would respire about 0.015 mg. of carbon dioxide per hour. At this rate the maximum number of leafhoppers per leaf at any time in the course of the 2½-hour determination would not respire enough carbon dioxide to interfere with the photosynthesis measurements. Thus, the insects were permitted to feed continuously even during the determinations and were confined to the part of the leaf surface included by the leaf cup rather than to all the surface included in a sleeve cage.

The data in table 3 and figure 3, *C*, show that at a point in the experiment corresponding to May 17 in the field, photosynthesis was reduced 16.5 percent and transpiration 4.9 percent. At a point corresponding to June 25 the reductions were, respectively, 19.2 and 13.8 percent, and by July 26 they were 27.1 and 12.9 percent. It would appear from these data that before mid-July, when the insect population is still rather low, marked reductions may occur in the rate of photosynthesis, especially in the early part of the period. The gradual reduction in photosynthesis recorded from week to week in experiment C was not significant, but the final reduction at a point corresponding to July 26 was highly significant (see fig. 4, *C*, *a*, for apparent injury toward end of experiment). The reduction in transpiration was not significant. In experiment D (table 4 and fig. 3, *D*), which was carried only to a point corresponding to June 5 in the field, reductions in transpiration and photosynthesis, although not significant, were recorded.

## EXPERIMENT E

Table 5 and figure 3, *E*, show the results of a fifth experiment with the various species of apple leafhoppers. This experiment yielded data similar to those in the experiments described above. Three feeding periods with a population of approximately 25 leafhoppers per leaf brought about a reduction in the rate of photosynthesis of 23.8 percent and in transpiration of 16.5 percent. The reduction in transpiration over the entire period of the experiment was significant; the reduction in photosynthesis was significant from one feeding period to the next and highly significant over the 3-week period.

TABLE 3.—The effects of apple leafhopper injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment C

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)		Test Check ×100	Ex-pected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)		Test Check ×100	Ex-pected rate <sup>2</sup>
		Check	Test				Check	Test		
	Milli-grams	Milli-grams	Milli-grams		Per-cent <sup>2</sup>	Grams	Grams	Grams		Per-cent
May 25	17.0	14.3	24.6	172.0		0.212	1.49	1.97	132.2	
May 26	16.3	14.5	20.1	138.6		.200	1.56	2.06	132.1	
May 27	17.4	14.8	24.6	166.2		.224	1.35	1.86	137.8	
May 28	16.8	15.3	23.9	156.2		.215	1.51	2.07	137.1	
May 29	14.8	11.0	18.0	163.6		.217	1.39	1.78	134.3	
Average				159.3					133.5	
May 30	15.2	19.0	28.5	150.0	94.2	.212	1.69	1.99	117.8	88.2
May 31	15.5	20.3	26.0	128.1	80.4	.191	1.40	1.82	130.0	97.8
June 1	14.8	17.3	23.0	133.0	83.5	.185	1.56	1.90	121.8	91.2
June 2	14.9	18.8	25.7	136.7	85.8	.175	1.64	2.31	140.9	105.5
June 3	16.4	18.4	24.6	133.7	84.0	.182	1.69	1.96	116.0	86.9
June 4	17.3	16.3	25.6	157.1	98.7	.189	1.63	1.85	113.5	85.0
June 5	15.5	13.4	21.2	158.2	99.3	.233	1.44	1.66	115.3	86.4
June 7	17.6	18.7	25.8	138.0	86.7	.215	1.35	1.50	111.1	83.2
June 8	16.2	14.4	20.6	143.1	89.9	.573	1.12	1.79	159.8	119.7
June 9	17.7	21.0	29.1	138.6	87.0	.192	1.53	2.18	142.5	106.7
June 10	17.8	17.1	26.1	152.6	95.8	.181	1.55	2.03	131.0	98.3
June 11	17.9	34.9	43.4	124.4	78.1	.185	1.64	2.18	132.9	99.5
June 15	16.6	11.5	15.6	135.7	85.2					
June 16	16.6	15.2	15.7	103.3	64.9					
June 17	17.7	16.6	22.9	138.0	86.7					
June 18	17.1	13.2	22.1	167.4	105.1					
June 19	16.5	10.2	13.4	131.4	82.5	.385	1.38	1.70	123.2	92.3
June 20	16.5	11.2	14.9	133.0	83.5	.279	3.83	4.10	107.0	80.1
June 21	18.4	19.9	23.4	117.6	73.9	.405	1.23	1.53	119.5	89.5
June 22	15.7	13.7	14.3	104.4	65.6	.406	1.54	2.02	131.2	98.3
June 23	15.2	10.7	12.3	115.0	72.2	.430	1.31	1.68	128.2	96.0
June 24	15.1	5.9	8.5	144.1	90.5	.418	1.09	1.35	123.9	92.8
June 25	15.0	9.5	11.5	121.1	76.1	.409	1.21	1.42	117.4	87.9
June 27	15.9	16.0	15.6	97.5	66.8	.414	1.23	1.44	117.1	87.1
June 28	15.1	8.3	11.6	139.8	95.8	.414	1.28	1.26	98.4	79.4
June 29	15.3	12.4	13.3	107.3	73.5	.415	1.27	1.27	109.4	88.3
June 30	15.9	7.0	8.8	125.7	86.1	.415	1.16	1.32	113.8	91.8
July 1	17.1	10.3	9.7	96.1	65.8	.504	1.18	1.19	100.8	81.4
July 2	14.4	11.0	11.0	106.0	68.5	.316	1.03	.95	92.2	82.7
July 3	17.0	13.7	18.8	137.2	87.7	.403	2.08	2.35	113.0	88.4
July 8	16.1	7.2	11.6	161.1	102.9	.459	1.25	1.66	132.8	104.1
July 9	16.7	10.6	12.9	121.7	77.8	.464	1.25	1.50	122.0	95.6
July 10	18.3	12.5	16.0	128.0	81.8					
July 11	16.5	10.0	14.1	141.0	90.1					
July 13	16.8	11.7	11.6	99.1	63.3					
July 14	17.4	13.4	17.3	129.1	82.5					
July 15	21.1	15.2	22.7	149.3	95.4					
July 16	15.0	7.4	8.8	118.9	76.0					
July 17	15.8	9.5	8.6	90.5	57.8					
July 18	18.2	11.2	11.5	102.7	65.6					
July 19	21.7	14.2	12.4	87.3	55.8					
July 20	18.4	10.5	15.6	148.6	95.0	.404	1.21	1.37	113.2	88.7
July 23	16.4	10.7	12.1	113.0	80.8					
Average				127.9	81.8				120.5	92.2

ANALYSIS OF VARIANCE OF  $\frac{\text{TEST}}{\text{CHECK}} \times 100$ 

Item	Degrees of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis.....	1	4,407.5	4,407.5	10.93	Highly significant.
	46	18,548.8	403.2		
Transpiration.....	1	870.1	870.1	1.65	Not significant.
	31	44,461.7	1,434.3		

<sup>1</sup> Leafhoppers were permitted to feed (field concentration) continuously even during the determinations.  
<sup>2</sup> See footnote 2, table 1, for method of calculation.

TABLE 4.—The effects of apple leafhopper injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment D

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)		Test Check × 100	Ex-pected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)		Test Check × 100	Ex-pected rate <sup>2</sup>
		Check	Test				Check	Test		
	<i>Milli-grams</i>	<i>Milli-grams</i>	<i>Milli-grams</i>		<i>Per-cent</i> <sup>2</sup>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>		<i>Per-cent</i>
June 15.....	16.1	17.3	22.3	128.9	-----	0.177	1.77	2.20	124.3	-----
June 16.....	15.6	15.0	19.2	128.0	-----	.207	1.67	1.79	107.2	-----
June 17.....	17.7	19.3	23.2	120.2	-----	.234	1.72	1.82	105.8	-----
June 18.....	16.1	17.7	22.7	128.2	-----	.201	1.63	1.76	108.0	-----
June 20.....	16.5	16.7	21.5	128.7	-----	.389	2.40	2.50	104.2	-----
June 21.....	-----	-----	-----	-----	-----	.357	2.61	2.80	107.3	-----
June 23.....	-----	-----	-----	-----	-----	.371	2.34	2.61	111.5	-----
June 24.....	14.5	13.8	17.3	125.4	-----	.450	1.72	1.80	104.7	-----
June 25.....	14.7	11.3	13.7	120.2	-----	.374	2.04	2.30	112.7	-----
Average.....	-----	-----	-----	125.7	-----	-----	-----	-----	109.3	-----
June 28.....	13.9	17.8	17.2	96.6	76.8	.377	2.16	2.48	114.8	105.6
June 29.....	-----	-----	-----	-----	-----	.401	2.07	2.35	113.5	104.4
June 30.....	14.9	20.7	24.9	120.3	95.7	.385	1.88	2.15	114.4	105.1
July 1.....	-----	-----	-----	-----	-----	.324	1.59	1.82	114.5	105.2
July 2.....	15.2	12.1	15.6	128.9	102.6	.379	1.96	1.93	98.5	90.7
July 3.....	14.3	15.9	17.1	107.6	85.6	.371	1.81	2.21	122.1	112.2
July 8.....	15.8	9.7	13.4	138.1	109.9	.404	1.92	2.35	122.5	112.5
July 9.....	14.9	10.3	9.6	93.2	74.1	.408	1.92	1.82	94.8	87.1
July 11.....	14.8	10.6	13.6	128.3	102.1	-----	-----	-----	-----	-----
July 12.....	-----	-----	-----	-----	-----	.367	1.87	1.96	104.8	96.3
July 13.....	16.2	10.1	12.2	120.8	96.2	.335	2.48	2.54	102.4	94.1
July 14.....	16.1	12.8	15.5	121.1	96.4	.313	2.09	2.18	104.3	95.9
July 15.....	14.4	9.6	10.1	105.2	87.7	.342	1.82	1.99	109.3	100.4
July 16.....	14.6	10.9	13.6	124.2	103.8	.334	2.14	2.12	99.1	84.5
July 17.....	14.9	11.9	9.9	83.2	65.1	-----	-----	-----	-----	-----
July 18.....	15.0	12.8	16.0	125.0	97.8	.236	2.01	2.03	101.0	97.0
July 20.....	14.0	12.6	13.3	105.6	82.7	.350	1.90	1.79	94.2	90.4
July 21.....	14.0	8.0	8.9	111.3	87.1	.348	1.67	1.70	101.8	97.7
July 22.....	14.2	13.3	10.0	75.2	58.9	.332	1.86	1.70	91.4	87.7
July 23.....	15.4	10.4	13.1	126.0	98.7	.312	1.96	1.49	76.0	73.0
Average.....	-----	-----	-----	112.8	89.5	-----	-----	-----	99.1	96.7

ANALYSIS OF VARIANCE OF  $\frac{\text{TEST}}{\text{CHECK}} \times 100$ 

Item	Degrees of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis.....	1	868.3	868.3	3.85	{Reduction, but not significant.
Transpiration.....	22	4,960.1	225.5		
	1	208.2	208.2	2.36	{Reduction, but not significant.
	26	2,292.6	88.2		

<sup>1</sup> Leafhoppers were permitted to feed (field concentration) continuously even during the determinations.<sup>2</sup> See footnote 2, table 1, for method of calculation.

TABLE 5.—The effects of apple leafhopper injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment E

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)			Test/Check × 100	Expected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)			Test/Check × 100	Expected rate <sup>2</sup>
		Check	Test					Check	Test			
	Milli-grams	Milli-grams	Milli-grams			Per-cent <sup>2</sup>	Grams	Grams	Grams			Per-cent
Aug. 2.....	16.9	30.1	32.3	107.3			0.416	3.11	2.90		93.2	
Aug. 3.....	15.5	18.6	19.8	106.5			.384	2.43	2.42		99.6	
Aug. 4.....	15.9	24.9	26.2	105.2			.405	2.15	1.88		87.8	
Average.....				106.3							93.4	
Aug. 14.....	16.4	25.2	23.4	92.9	87.4		.416	2.82	2.24		79.4	85.0
Aug. 15.....	15.9	25.1	24.8	98.8	93.0		.452	2.97	2.58		86.9	93.0
Aug. 18.....	16.1	18.4	17.4	94.6	89.0		.386	3.28	3.15		96.0	102.7
Average.....				95.4	89.8						87.4	93.6
Sept. 1.....	18.1	15.5	10.6	68.4	64.4		.340	2.84	2.61		91.9	98.3
Sept. 2.....	16.8	19.6	11.5	58.7	55.2		.332	2.46	1.88		76.4	81.7
Sept. 3.....	21.1	18.5	15.5	83.8	78.9		.410	2.45	1.87		76.3	81.6
Average.....				70.3	66.2						81.5	87.2
Sept. 9.....							.436	1.81	1.30		71.8	76.8
Sept. 10.....	16.5	12.3	12.2	99.2	93.3		.427	2.54	2.06		81.1	86.8
Sept. 11.....	15.9	15.6	9.8	62.8	49.5		.434	2.23	1.69		75.8	81.1
Average.....				81.0	71.3						77.9	81.6

## ANALYSIS OF VARIANCE OF TEST/CHECK × 100

Item	Degrees of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis.....	1	1,249.8	1,249.8	11.76	Highly significant.
	9	956.4	106.3		
Transpiration.....	1	277.8	277.8	5.26	Significant.
	10	528.6	52.9		

<sup>1</sup> Between each series of measurements the leafhoppers were permitted to feed.  
<sup>2</sup> See footnote 2, table 1, for method of calculation.

## EFFECTS ON PHOTOSYNTHESIS AND TRANSPIRATION OF APPLE LEAVES CAUSED BY FEEDING OF THE POTATO LEAFHOPPER

## EXPERIMENT F

The potato leafhopper (*Empoasca fabae* (Harr.)) is among the least common of the species that occur in the orchard, but the injury that it causes is easily identified. The typical feeding puncture is made in the vascular system of the leaf or stem and this usually results in blocking the conducting tissues (11). The leaf area distal to the point of injury becomes pale green in a triangular area bounded by veins and the leaf margin. Only mature leaves were used in this experiment to avoid the typical curling that results from the feeding of *E. fabae* on leaves that have not reached full size.

In a typical experiment about 10 to 15 leafhoppers per leaf were released in the cheesecloth cage over the apple shoot, after a relation-



ship in metabolism had been established between the two groups of leaves. The insects were permitted to feed for a week. Apparent photosynthesis was reduced 29.1 percent, but there was no significant effect on transpiration (table 6 and fig. 3, *F*). Two additional feeding periods were accompanied by a reduction in the rate of photosynthesis of 31.1 percent and in transpiration of 33.6 percent. At this time the leaves exhibited the characteristic pale-green areas as the only external symptom of injury. The data for transpiration and photosynthesis were found to be significant and highly significant, respectively.

TABLE 6.—The effects of potato leafhopper (*Empoasca fabae* H.) injury on the rates of photosynthesis and transpiration of Stayman Winesap apple leaves, experiment F

Date of leaf activity measurement <sup>1</sup>	CO <sub>2</sub> per cu. ft. of air	Photosynthesis (CO <sub>2</sub> per 100 cm. <sup>2</sup> per hour)		Test Check $\times 100$	Ex-pected rate	H <sub>2</sub> O per cu. ft. of air	Transpiration (H <sub>2</sub> O per 100 cm. <sup>2</sup> per hour)		Test Check $\times 100$	Ex-pected rate <sup>2</sup>
		Check	Test				Check	Test		
	Milli-grams	Milli-grams	Milli-grams		Per-cent <sup>2</sup>	Grams	Grams	Grams		Per-cent
July 29.....	13.5	16.1	20.1	124.8	-----	0.533	3.85	3.83	99.5	-----
July 30.....	15.5	19.0	23.3	122.6	-----	-----	-----	-----	-----	-----
Aug. 1.....	15.6	16.4	16.6	101.2	-----	.405	2.18	2.48	113.8	-----
Aug. 2.....	15.7	12.0	14.6	121.7	-----	.393	1.22	1.71	140.2	-----
Aug. 5.....	13.8	19.4	20.2	104.1	-----	.248	5.31	6.64	125.0	-----
Average.....	-----	-----	-----	114.9	-----	-----	-----	-----	119.6	-----
Aug. 14.....	15.9	13.0	9.6	73.8	64.2	.501	2.47	2.80	113.1	94.8
Aug. 15.....	14.8	13.9	11.1	79.9	69.5	.463	1.90	2.83	148.9	124.5
Aug. 18.....	15.8	15.0	13.6	90.7	78.9	.575	2.48	3.02	121.8	101.8
Average.....	-----	-----	-----	81.5	70.9	-----	-----	-----	128.0	107.0
Aug. 31.....	14.6	15.3	8.4	54.9	47.8	.335	3.60	3.22	89.4	74.7
Sept. 1.....	13.2	15.9	10.9	68.6	59.7	-----	-----	-----	-----	-----
Sept. 2.....	-----	-----	-----	-----	-----	.361	2.54	2.79	109.4	91.5
Sept. 3.....	16.2	15.9	12.5	78.6	68.3	.370	3.54	3.04	85.9	71.3
Average.....	-----	-----	-----	79.2	58.6	-----	-----	-----	78.4	87.4

#### ANALYSIS OF VARIANCE OF TEST CHECK $\times 100$

Item	Degree of freedom	Sum of squares	Mean of squares	F	Remarks
Photosynthesis.....	1	4,856.1	4,856.1	43.8	Highly significant.
	12	1,331.0	110.9		
Transpiration.....	1	1,020.0	1,020.0	1.68	Not significant.
	11	6,684.2	607.7		

<sup>1</sup> Between each series of measurements leafhoppers were permitted to feed.

<sup>2</sup> See footnote 2, table 1, for method of calculation.

#### EFFECTS ON INTERNAL STRUCTURE OF APPLE LEAVES CAUSED BY THE FEEDING OF APPLE LEAFHOPPERS

Microscopic examination of moderately injured leaves revealed small groups of empty palisade cells, often with one or two uninjured cells in the center of each group. The cell walls appeared intact, although minute punctures through which cell contents were removed probably were present. Figure 5 shows cross sections of two apple leaves from which the cell contents of the upper palisade layer have been removed. Figure 5, *B*, shows an apple leafhopper killed, embedded, and sectioned in situ. The proboscis was lost in sectioning, but empty cells in the palisade layer show where the insect was feeding. During the progress

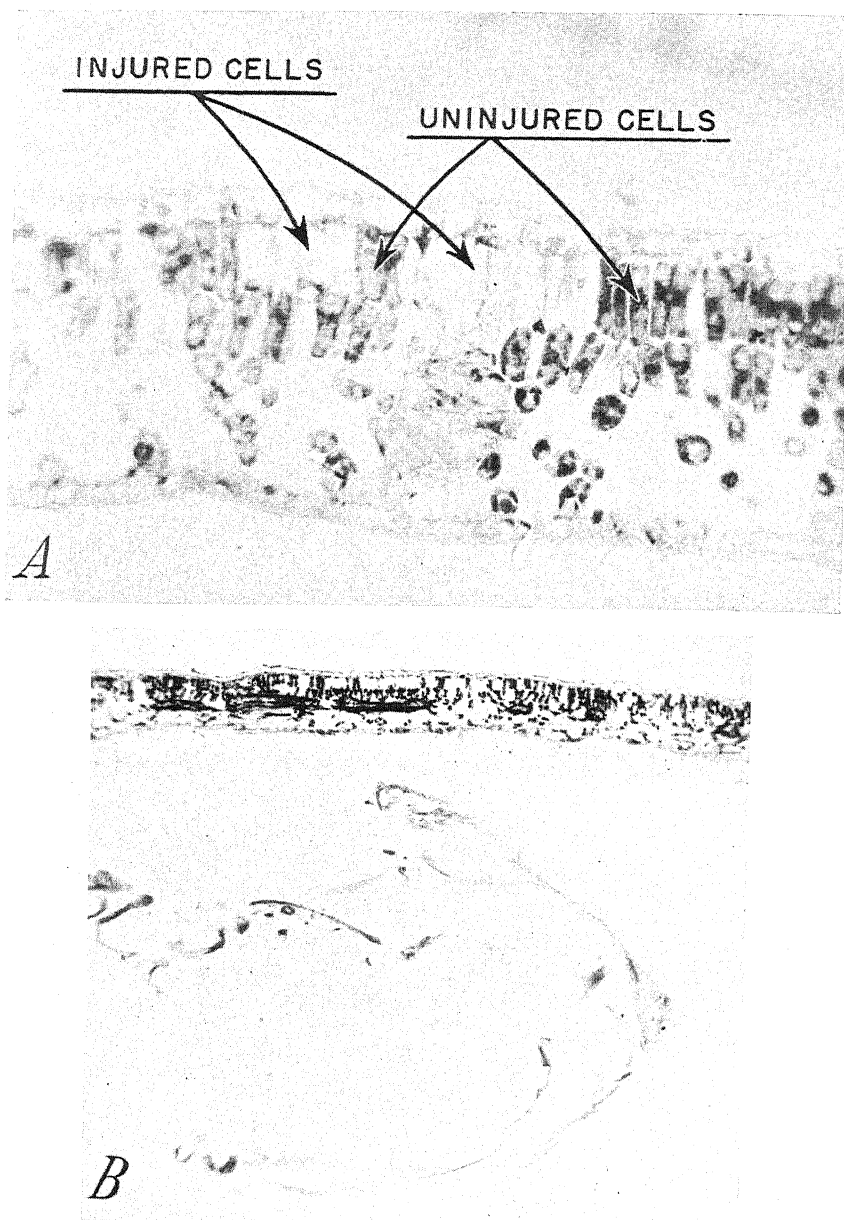


FIGURE 5.—Cross sections of Stayman Winesap apple leaves showing white apple leafhopper (*Typhlocyba pomaria*) feeding injury: *A*, Injured and uninjured cells of the palisade layer; *B*, cross section of leafhopper head in feeding position on under side of leaf, showing relative size of the insect and the cells upon which it has fed.

of these investigations a paper was published (11) in which somewhat similar results with apple leaves were reported. Smith and Poos (12) have pointed out that a small group of uninjured cells is often found in the center of a group of several injured ones where a leafhopper has been feeding.

Leafhopper injury caused a reduction in the thickness and fresh weight of apple leaves. Several hundred leaves were weighed and examined under the microscope in the field. Severely injured Black Twig leaves weighed approximately one-half as much (0.063 gm.) per unit area as uninjured leaves (0.123 gm.); they were from 0.097 to 0.120 mm. thick as compared with a thickness of 0.149 to 0.193 mm. in uninjured leaves.

#### DISCUSSION

In most instances considerable stippling was evident on the upper surfaces of the leafhopper-injured leaves before appreciable reductions in apparent photosynthesis and transpiration were recorded. A study of cross sections of injured leaves (fig. 5) shows that the spongy mesophyll tissue, in which probably a large part of the photosynthetic activity is carried on, is not affected until the palisade layers are markedly injured. When injured leaves are viewed from beneath by transmitted light, it is apparent that more light passes through the small injured spots than through the uninjured areas. It can be assumed, then, that that part of a leaf (spongy mesophyll cells) immediately under an injured area (palisade cells) receives more light after injury than it did before and conditions may be more favorable for photosynthesis in this region.

A given number of potato leafhoppers had a more detrimental effect on the metabolic activity than an equal number of leafhoppers of other species; this is in agreement with the data of Smith and Poos (12). In almost every case the leafhopper injury, whether of the vein or mesophyll type, affected the rate of photosynthesis more than the rate of transpiration, and usually sooner. An examination of the injured leaves showed that the moderately injured areas were low in active chlorophyll, although in other respects they appeared normal, at least morphologically. Thus, while photosynthesis might be reduced in these areas because of relatively low chlorophyll content, the loss of water vapor could go on at a rate more nearly normal. Severe injury by the potato leafhopper (*Empoasca fabae*) or by apple or grape leafhoppers (*Typhlocyba* spp. and *Erythroneura* spp.) ultimately resulted in a reduction in transpiration, associated with a blocking of the vascular tissues in the case of *Empoasca* (11, 12, 13),<sup>6</sup> and a breakdown of the mesophyll cells in the case of the other two (5, 11, and others).<sup>6</sup>

The decrease in leaf weight and thickness associated with leafhopper injury evidently results from the removal of the cell contents and the eventual drying and partial collapse of the injured cells. The total number of cells is probably not affected by leafhopper injury on a given leaf, as the leaf is a primary body; however, it is assumed that reduced vigor of the infested plant brought about by continued high populations of leafhoppers will eventually result in smaller leaves and other plant parts, especially the fruits.

<sup>6</sup> See footnote 4.

The question may arise as to which is more important to the welfare of the leaf—reduction in photosynthesis and transpiration caused by spray materials used to control the leafhopper, or injury from the insect itself. In practically all of the writers' experiments with nicotine sprays (data unpublished) there has been a reduction in photosynthesis and transpiration for a day or so after spray applications, and then recovery. However, the data on leafhopper injury presented here show that a leaf does not recover its original status in respect to apparent photosynthesis once it has been even moderately injured. This fact is of special importance where early season leafhopper injury occurs on leaves. Cell structure and the capacity of the leaf to function properly are adversely affected for the remainder of the season. This emphasizes the importance of early control of leafhoppers, both from the standpoint of reducing later populations of the insects and of obviating the permanent effects that they may have on leaf metabolism.

#### SUMMARY AND CONCLUSIONS

Injury to Stayman Winesap apple leaves caused by several species of apple leafhoppers (*Typhlocyba* spp.), grape leafhoppers (*Erythroneura* spp.), and the potato leafhopper (*Empoasca fabae*) was accompanied by a more or less marked reduction in apparent photosynthesis and transpiration. Photosynthesis usually was affected sooner and to a greater extent than transpiration.

A given number of potato leafhoppers had a more detrimental effect on leaf metabolism than an equal number of apple or grape leafhoppers.

Cross sections of injured leaves showed that the mesophyll-feeding types of leafhopper (*Typhlocyba* spp. and *Erythroneura* spp.) removed the contents of the cells in the palisade layers, while the spongy mesophyll cells were not significantly affected unless the leaf had been severely injured.

The data show that apparent photosynthesis and transpiration of apple leaves may be reduced early in the growing season when the leafhopper population is moderately low, and when this occurs the capacity of the injured leaves to function normally is permanently impaired. Early control of these insects is therefore important.

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## EFFECT OF EROSION ON FERTILITY CHANGES IN THE SHELBY LOAM PROFILE<sup>1</sup>

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### INTRODUCTION

It is generally accepted that under virgin conditions the soil is never completely depleted of its fertility, but tends to reach a level of fertility in equilibrium with that of its environment (1).<sup>3</sup> The supply of nutrients is adequate, therefore, for the native vegetation. Soil-fertility-depleting factors, such as cultivation, are absent. Erosion is at a minimum.

The effects of most farming operations, with the commonly attendant removal of soil by erosion, have in too many instances decreased the once relatively large amount of nutrients in the soil. Observations suggest, also, that all too often farmers have used management practices that they believed would maintain or increase the productivity of their soil, when such practices were failing to achieve this purpose.

Data obtained at the Soil Conservation Experiment Station, Bethany, Mo., show that, under similar conditions of soil, slope, and climate, the rate of soil removal is dependent largely upon the crop and cultural treatment.<sup>4</sup> Each treatment affords its own degree of protection against the erosional action of water. Crop and management practices also cause chemical, physical, and biological effects on the soil that change the original soil properties.

Accurate soil- and water-loss records from several plots have been kept at the Bethany station for a number of years. Soil-fertility analyses of these data have been made to learn how soil- and crop-management practices may affect soil losses or losses and gains in soil fertility. The results of these preliminary studies dealing with soil and water losses under different crop and cultural treatments are presented here, as such losses reflect the changes in fertility brought about by them.

### PLAN OF STUDY

Soil samples were taken from plots of series 1 of the Bethany station, which are maintained for comparative studies of various soil- and crop-management practices. The cropping system, soil and water losses, and other pertinent data are given in table 1. The plots were prepared in 1930, and the records for a complete crop year were begun on January 1, 1931, so that effects during 7 years have been measured.

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<sup>2</sup> Acknowledgment is made to Dr. W. A. Albrecht, chairman, Soils Department, University of Missouri, for helpful suggestions and for guidance during the course of this study and in the preparation of this report.

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 297.

<sup>4</sup> WOODRUFF, C. M., and SMITH, D. D. PROGRESS REPORT OF THE PROBLEM AREA OF SHELBY LOAM AND RELATED SOILS, SOIL AND WATER CONSERVATION EXPERIMENT STATION, BETHANY, MO., 1930-35. U. S. Soil Conserv. Serv. ESR-5, 180 pp., 1938. [Processed.]

TABLE 1.—Average annual soil and water losses for a 7-year period (1931-37), Shelby silt loam, plot series 1, Bethany, Mo.

Plot No. <sup>1</sup>	Crop	Pre- cipita- tion	Surface run-off			Soil loss, run-off per acre		Period required for 7-inch surface erosion <sup>2</sup>
			Quan- tity	Percent- age of precipita- tion	Density per acre-inch			
1.....	Corn, annually.....	Inches	Inches	Percent	Tons	Tons	Inches	Years
2.....	do.....	31.45	9.07	28.84	7.32	66.35	0.44	15.1
3, 4, 5.....	Rotation untreated <sup>3</sup> .....	31.45	9.15	29.09	5.80	53.03	.35	18.9
6.....	do <sup>3</sup> .....	31.45	5.11	16.25	1.72	8.81	.06	113.5
7.....	Rotation, fertilized <sup>4</sup> .....	31.45	4.98	15.83	1.04	5.18	.04	-----
8.....	Alfalfa, fertilized <sup>5</sup> .....	31.45	3.96	12.59	.93	3.69	.03	-----
9.....	Bluegrass.....	31.45	2.50	7.95	.08	.20	.001	3,000
10.....	Surface fallow.....	31.45	3.06	9.73	.07	.22	.001	4,545
11.....	Subsoil fallow <sup>6</sup> .....	31.45	9.28	29.51	9.28	86.15	.58	11.6
			9.32	29.63	6.46	60.25	.40	16.6

<sup>1</sup> Plot 1, length, 145.2 feet; 0.02 acre. Other plots, length, 72.6 feet; 0.01 acre. All plots, width, 6.0 feet; slope, 8 percent.

<sup>2</sup> Weight of plow-depth soil taken as 2 million pounds per acre.

<sup>3</sup> Rotation: Corn, wheat (clover-timothy), clover-timothy.

<sup>4</sup> Lime, 3 tons per acre, 1930; 20-percent superphosphate, 250 pounds per acre on wheat. Rotation: Corn, wheat (clover-timothy), clover-timothy.

<sup>5</sup> Lime, 3 tons per acre, 1930; 20-percent superphosphate, 250 pounds per acre, every 3 years.

<sup>6</sup> In 1930, 7 inches of surface soil removed.

In order that the changes in fertility through the removal of soil by erosion from the different plots under various crop and cultural treatments might be compared on some basis, the study included an analysis, obtained in 1939 from a plot continuously in bluegrass since 1930, of 1-inch horizons of a 0- to 13-inch profile. Since the amount of soil lost from this plot has been negligible during the time of the experiment, and since this plot simulates virgin conditions, it was taken as a basis for comparing the amount of soil lost and for evaluating the gain or loss in fertility of the other plots. Comparisons could be made of the 0- to 7-inch horizon of any plot according as it corresponded to 7 successive 1-inch horizons of the 0- to 13-inch profile.

Originally all the plots had the same depth of surface soil, and therefore the equivalent horizons of plots for comparing with the continuous bluegrass plot could be obtained by taking into consideration the amount of surface soil, in inches, that had been removed by erosion. A 13-inch depth was chosen arbitrarily as sufficient, because this depth extended into the subsoil or B horizon of the Shelby soil, and because soil removal on none of the plots had exceeded this depth. In general, this method of approach conforms to the practice followed in making conservation surveys (8). Characteristics of profiles undisturbed by erosion were used as a basis from which to determine the degree of erosion that had occurred on truncated soil profiles.

Another reason for extending the sampling to include part of the subsoil is the fact, brought out by conservation surveys, that many of the Shelby soil areas now are being farmed on or near subsoil levels. Additional information is needed in order to treat the subject of subsoil farming more intelligently. Soil- and crop-management practices advocated for comparatively noneroded soils apparently have not met with success when applied indiscriminately to more or less desurfaced soils.



Further information pertaining to the plots is contained in a report from the Bethany<sup>5</sup> station, and various chemical and physical characteristics of the Shelby loam profile and the soil on each plot are reported in other publications of the Department of Agriculture (6, 7, 10).

Annual samples, representing a composite of seven borings for each plot, taken by beginning 5 feet from the top of the slope and extending downward at 10-foot intervals, were obtained each fall from the surface 7-inch layer of the plots under study. Plot 1, in continuous corn, was twice the length of the other plots, and was divided equally into upper and lower sections. Each section was sampled like the other plots. Plot 8, in continuous bluegrass, which was used as the basis for comparisons with the other plots, was sampled during the spring of 1939 by 1-inch horizons to a 13-inch depth. The seven borings on this plot for each 1-inch horizon were composited. These samples of limited horizons measure the changes by 1-inch surface units that would occur in the profile with progressive erosion.

To obtain a measure of the changes in fertility of the plots, the successive 1-inch profile samples (0 to 13 inches) taken on plot 8, in 1939, and the annual composite surface samples (0 to 7 inches) taken in 1931, 1933, 1935, and 1937 on the other plots, were analyzed for their total exchange capacity, exchangeable calcium, exchangeable magnesium, total carbon content, total nitrogen content, and hydrogen-ion concentration. Ammonium acetate was used as the leaching agent for the base-exchange determinations. Exchangeable calcium and magnesium in the leachate were determined by standard analytical methods. The total carbon content was measured by dry combustion and converted to organic-matter percentages by using the conventional Wolff factor, 1.724. The Kjeldahl method was used in determining total nitrogen. The pH was measured with a quinhydrone electrode.

#### EXPERIMENTAL RESULTS

The results may be shown most conveniently by presenting first the data from the profile of plot 8, continuously in bluegrass and with insignificant erosion. The properties of the successive 1-inch horizons of this noneroded profile will serve as a basis for comparing the properties of the profiles of the other plots under their different treatments. Any differences will correspond to greater or lesser amounts of erosion and truncation of the profiles of these plots.

##### PROFILE CHARACTERISTICS OF THE NONERODED SOIL

Characteristics determined for the 1-inch horizons for the 0- to 13-inch profile on the noneroded bluegrass plot (plot 8) included total exchange capacity, exchangeable calcium, exchangeable magnesium, pH, organic matter, and nitrogen. The detailed data are given in table 2 and presented graphically in figures 1 and 2. Perhaps the most noticeable fact in the data, considered as a whole, is the similarity in properties of the first six or seven successive 1-inch horizons.

<sup>5</sup> See footnote 4.

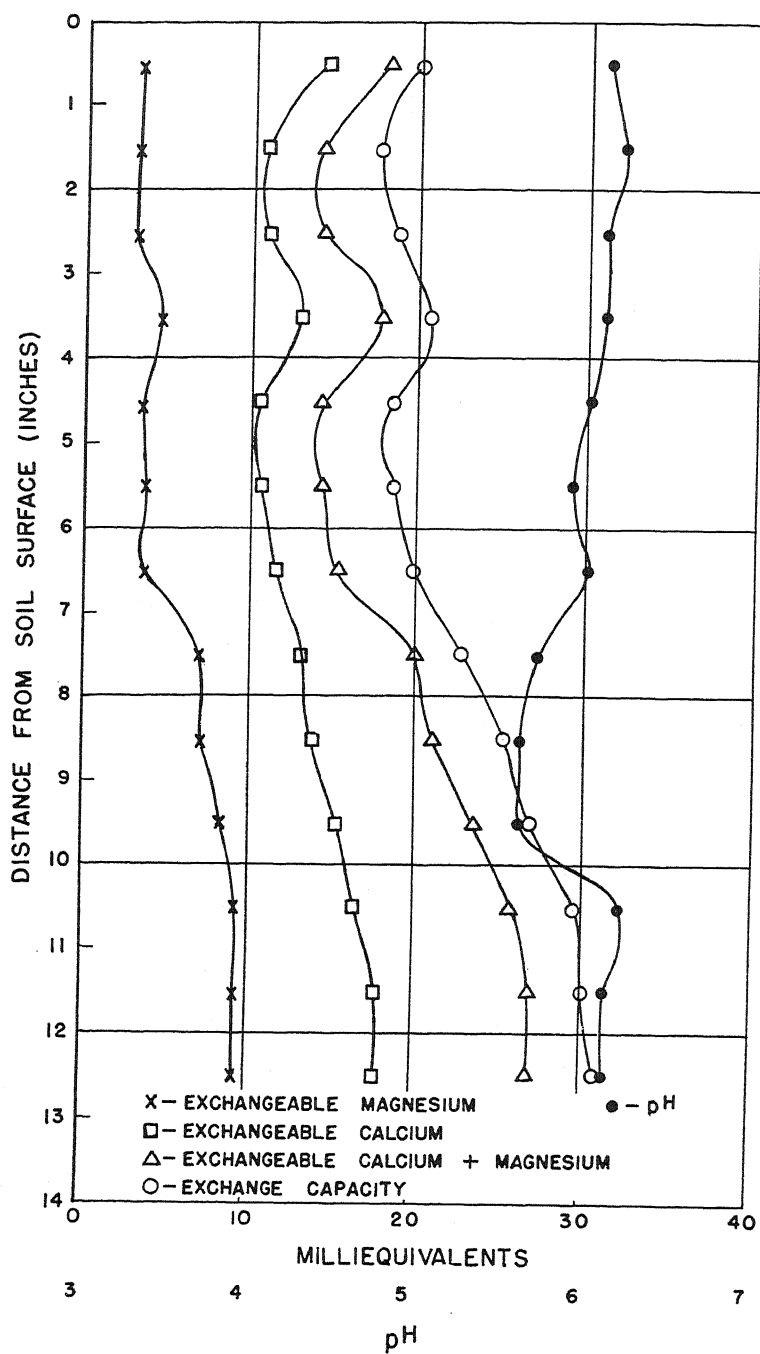


FIGURE 1.—Exchangeable calcium and magnesium, total exchange capacity, and of the soil at different levels in the profile of the noneroded bluegrass plot.

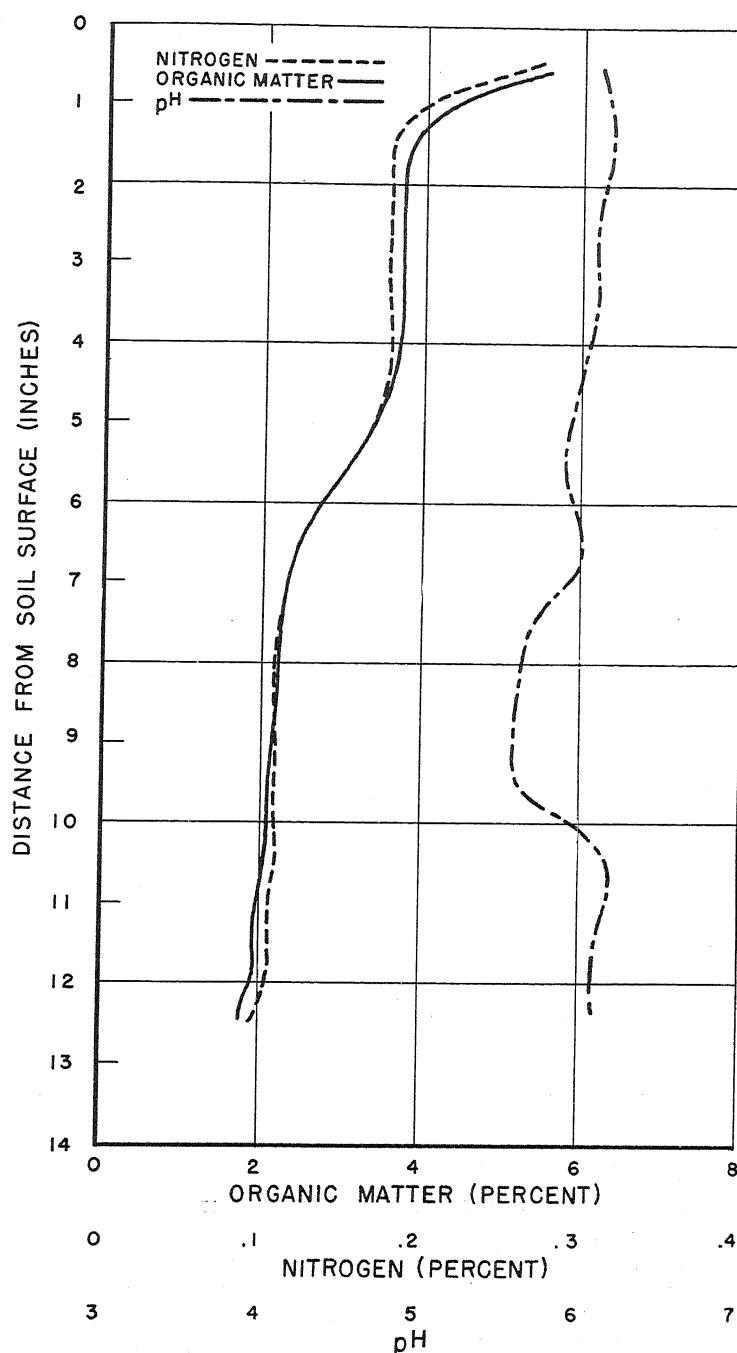


FIGURE 2.—Nitrogen, organic matter, and pH of soil at different levels in the profile of the noneroded bluegrass plot.

TABLE 2.—Chemical properties of bluegrass plot profile, 1939, Shelby silt loam

Profile depth (inches)	Total ex- change capacity	Exchangeable calcium		Exchangeable magnesium		Total exchange- able calcium- magnesium		Organic matter	Nitro- gen	C/N ratio	pH
		Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>	Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>	Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>				
0-1.....	20.10	14.60	72.64	3.68	18.31	18.28	90.95	5.71	0.270	12.28	6.1
1-2.....	17.80	10.93	61.40	3.37	18.93	14.30	80.34	3.82	.181	12.24	6.2
2-3.....	18.77	11.02	58.71	3.34	17.79	14.36	76.51	3.75	.179	12.14	6.1
3-4.....	20.65	12.98	62.86	4.75	23.00	17.73	85.86	3.71	.178	12.08	6.1
4-5.....	18.48	10.63	57.52	3.59	19.43	14.22	76.95	3.61	.177	11.84	6.0
5-6.....	18.53	10.56	56.99	3.72	20.08	14.28	77.06	3.09	.154	11.66	5.9
6-7.....	19.84	11.50	57.96	3.69	18.60	15.19	76.56	2.44	.121	11.67	6.0
7-8.....	22.54	12.94	57.41	7.00	31.06	19.94	88.46	2.29	.111	11.99	5.7
8-9.....	25.00	13.79	55.16	7.12	28.48	20.91	83.64	2.19	.110	11.53	5.6
9-10.....	26.49	15.04	56.78	8.31	31.37	23.35	88.15	2.10	.108	11.29	5.6
10-11.....	29.22	16.32	55.85	9.13	31.25	25.45	87.10	2.05	.107	11.14	6.2
11-12.....	29.73	17.57	59.10	9.04	30.41	26.61	89.51	1.94	.105	10.72	6.1
12-13.....	30.66	17.48	57.01	9.10	29.68	26.58	86.69	1.78	.097	10.66	6.1
0-7.....	19.17	11.75	61.29	3.73	19.46	15.48	80.75	3.73	.180	12.03	6.1
7-13.....	27.27	15.52	56.91	8.28	30.36	23.81	87.31	2.06	.106	11.26	5.9
0-13.....	22.91	13.49	58.88	5.83	25.45	19.32	84.33	2.96	.146	11.75	6.0

<sup>1</sup> Milliequivalents per 100 gm. of soil.<sup>2</sup> Percentage of total exchange capacity.

## TOTAL EXCHANGE CAPACITY, EXCHANGEABLE CALCIUM, AND EXCHANGEABLE MAGNESIUM

The total exchange capacity, exchangeable calcium, and exchangeable magnesium were fairly constant in the surface 7 inches of soil. This is due partly to the effect of soil mixing resulting from plowing prior to the time when the bluegrass was established on the plot. No explanation can be given at the present time for the increase in the 3- to 4-inch layer. The effect of organic matter on the base-exchange properties is shown in the 0- to 1-inch horizon. This horizon has a higher organic-matter content (5.71 percent), with a correspondingly higher amount of exchangeable calcium (14.60 milliequivalents per 100 gm. of soil) than the average organic-matter content (3.73 percent) and exchangeable calcium (11.75 milliequivalents) for the 0 to 7-inch horizon. The total exchange capacity and exchangeable magnesium increased less, proportionately, in the corresponding horizons. A gradual increase in magnitude occurred as the clay content increased with depth. Base saturation followed the same trend.

## PH VALUES

The pH values of the soil were fairly constant in the surface 7 inches. They decreased in the 7- to 10-inch horizons, and increased in the lower 3 inches to approximately the values of the surface 7 inches.

## ORGANIC MATTER AND NITROGEN

The organic matter and the nitrogen content both decreased decidedly at approximately the 6-inch level in the profile. An independent study of the depth of root penetration of different crops tested at the station showed that the mass of bluegrass roots did not penetrate much below 6 inches. This fact and the prior effects of plowing may account for the change.

The progressive accumulation of organic matter in the continuous bluegrass plot for the period 1931 to 1937 (table 3, plot 8), and the



TABLE 3.—Chemical properties of plots of series 1, Shelby silt loam

Plot No. and treatment	Year	Total ex- change capacity		Exchangeable cal- cium		Exchangeable mag- nesium		Total exchangeable calcium and mag- nesium		Organic matter	Nitrogen	C/N ratio	pH
		Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>	Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>	Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>	Milli- equiva- lents <sup>1</sup>	Percent <sup>2</sup>				
1-U; corn annually	1931	19.61	61.35	12.03	3.85	10.63	13.88	80.98	4.05	Percent	0.194	12.13	5.7
	1933	18.85	65.15	12.28	4.17	22.12	16.45	87.27	3.64			11.86	6.1
	1935	19.27	62.22	11.99	3.92	20.34	15.91	82.56	3.65			11.77	6.2
1-L; corn annually	1931	18.38	59.58	10.95	3.41	18.55	13.36	78.13	3.41			12.46	5.6
	1933	16.80	63.16	10.61	3.41	21.30	14.02	83.45	3.21			12.35	5.6
	1935	17.34	66.96	11.61	3.78	21.80	13.39	88.75	3.01			12.04	6.1
2; corn annually	1931	17.24	61.82	10.66	4.01	23.25	14.67	85.09	2.50			11.42	6.1
	1933	18.94	54.44	10.31	3.81	20.12	13.12	74.55	2.38			11.90	6.1
	1935	17.09	58.34	9.97	3.45	21.19	13.32	78.53	2.93			11.63	5.8
3; rotation, untreated <sup>3</sup>	1931	16.62	56.74	9.43	3.50	21.65	14.04	82.39	3.03			11.98	6.1
	1933	19.08	52.67	10.05	3.88	21.34	13.02	78.34	2.64			11.89	6.0
	1935	17.39	62.79	10.92	3.66	20.75	13.93	73.01	2.49			11.53	6.2
4; rotation, untreated <sup>3</sup>	1931	17.03	54.67	10.77	3.46	18.75	14.07	79.94	3.47			11.38	5.8
	1933	17.45	57.42	9.51	3.45	18.33	12.77	74.99	3.20			11.81	5.8
	1935	17.55	56.07	10.02	3.27	18.63	13.02	71.07	3.08			11.57	5.5
5; rotation, untreated <sup>3</sup>	1931	16.89	54.91	10.66	3.70	19.90	13.72	78.62	3.50			11.08	5.6
	1933	18.32	59.62	10.07	3.63	21.46	13.11	74.70	3.56			11.79	5.4
	1935	19.94	66.30	13.22	3.48	19.00	13.70	81.11	3.34			11.40	5.4
6; rotation, fertilized <sup>4</sup>	1931	18.35	58.09	10.66	3.54	17.75	16.76	84.05	3.43			12.12	5.8
	1933	18.03	58.58	10.58	2.97	16.10	13.63	74.28	3.61			12.15	5.7
	1935	17.98	56.56	10.17	3.40	18.83	13.98	77.41	3.59			11.40	5.5
7; alfalfa, fertilized <sup>4</sup>	1931	18.75	60.69	11.38	3.46	19.47	15.03	80.16	3.38			12.03	6.1
	1933	18.20	70.49	12.83	3.65	17.36	15.63	87.86	3.33			11.77	5.7
	1935	19.05	74.62	15.34	3.76	19.71	19.10	100.26	3.65			11.89	6.8
8; bluegrass	1931	18.95	58.10	11.01	3.20	17.07	17.67	91.70	3.57			11.82	6.1
	1933	19.17	64.79	12.42	4.01	21.16	15.02	93.25	3.79			12.40	5.5
	1935	20.02	87.71	15.56	3.27	17.66	15.09	81.85	3.91			11.96	5.5
9; surface fallow	1931	19.98	77.33	13.32	3.47	17.33	15.37	94.17	4.05			11.98	6.5
	1933	19.06	55.09	10.50	3.88	20.36	15.44	77.28	4.02			12.15	6.3
	1935	19.20	69.90	13.42	4.12	20.62	17.38	73.45	3.53			11.85	5.6
10; subsoil fallow <sup>6</sup>	1931	19.35	70.96	13.73	3.85	19.40	17.58	90.63	4.07			11.63	6.2
	1933	20.01	57.07	11.42	3.97	19.84	15.30	80.85	3.71			12.41	5.5
	1935	21.86	54.39	11.89	4.71	23.92	16.97	75.91	4.29			12.15	5.5
10; subsoil fallow <sup>6</sup>	1931	21.40	55.98	4.90	23.37	19.03	22.98	70.30	2.74			11.61	6.3
	1933	24.09	65.41	5.03	23.37	19.03	22.98	70.30	2.61			11.45	5.4
	1935	26.63	65.41	5.06	23.37	19.03	22.98	70.30	2.38			11.33	5.7
10; subsoil fallow <sup>6</sup>	1931	26.63	65.41	5.06	23.37	19.03	22.98	70.30	2.06			11.70	6.0
	1933	26.63	65.41	5.06	23.37	19.03	22.98	70.30	1.95			12.42	6.0
	1935	26.23	62.68	7.65	29.17	24.09	25.56	86.32	1.91			13.20	5.9
10; subsoil fallow <sup>6</sup>	1931	28.37	69.35	8.44	29.75	25.56	25.56	90.10	1.51			10.97	5.7
	1933	28.37	69.35	8.44	29.75	25.56	25.56	90.10	1.51			10.97	5.7
	1935	28.37	69.35	8.44	29.75	25.56	25.56	90.10	1.51			10.97	5.7

<sup>1</sup> Milliequivalents per 100 gm. of soil.<sup>2</sup> Percentage of total exchange capacity.<sup>3</sup> Rotation: Corn; wheat (clover-timothy); clover-timothy.<sup>4</sup> Lime, 3 tons per acre, 1930; 20-percent superphosphate, 250 pounds per acre on wheat.<sup>5</sup> Lime, 3 tons per acre, 1930; 20-percent superphosphate, 250 pounds per acre every 3 years.<sup>6</sup> In 1930, 7 inches of surface soil removed.

high base saturation of the exchange complex suggest that the calcification process of soil development might be operating under these environmental conditions. There may have been considerable leaching, but even so it was not enough to remove much of the carbonates to lower levels in the profile.

The process of leaching may have been retarded during this period especially during the last 2 years, a fact which might be attributable to the precipitation deficiency of 22 inches for the 7-year period. The normal annual precipitation, based on 50 years of records, obtained at the United States Weather Bureau Station at Bethany, is 34.44 inches. In 1936 and 1937 the annual precipitation was only 24.43 and 21.80 inches respectively.

The accompanying increase in nitrogen content suggests that conditions may have been favoring increased microbial activity of non-symbiotic nitrogen-fixing organisms. Definite conclusions on this point, however, are not warranted at this time, nor will they be until the microbial relationship of these plots is studied.

Albrecht and Smith (2) have shown that for the nonlegumes redbot and bluegrass, a soil completely saturated with calcium was more effective in the delivery of nitrogen from the supply in the soil to the crop than was a partly saturated soil. Since the percent base saturation for this plot (plot 8, table 3) increased from 77 in 1931 to 91 in 1937, and since environmental conditions suggest a minimum leaching of nitrogen, it appears that the relatively large increase in nitrogen found for the continuous bluegrass plot is not entirely fortuitous. It would seem that the nitrogen accretion can be attributed at least in part to an accumulation of the immobile nitrogen combined in the residue of plant and microbiological tissues.

Recent work has shown that nitrogen moves downward more rapidly in acid than in alkaline soil profiles (9); lime also has been found to flocculate a part of the organic nitrogen in solution. It appears that when there was a change in soil reaction from moderate acidity (pH 5.6) to nearer neutrality (pH 6.2), accompanied by a 25-percent increase in the calcium saturation of the exchange complex, the rate of nitrogen removal to lower horizons was reduced. The result was an increase in nitrogen content from 0.173 in 1931 to 0.205 percent in 1937.

The accumulation of organic matter has not only increased the nitrogen content, but its subsequent decomposition has set free all the other nutrients found in combination with it. Greater quantities of organic acids are released by the greater amount of decomposing organic matter. These, in turn, release greater stores of mineral nutrients, such as calcium, from the soil minerals. These combined effects, along with the increased microbiological activity, may explain in part the progressively larger amounts of exchangeable bases found under continuous bluegrass.

#### PROFILE CHARACTERISTICS OF THE ERODED SOILS

That truncation of a profile by erosion reduces the fertility content in measurable amounts is indicated by comparing the characteristics of the eroded plots with those of the noneroded profile under bluegrass. Plowing and cultivation of the surface soil, which bring up soil from deeper horizons, serve to complicate the measurement of the changes in the surface-soil characters caused by erosion. Observation and

evaluation of these disturbances in the profile are possible from the station studies.

#### TOTAL EXCHANGE CAPACITY

An excessive soil loss from the Shelby loam is reflected in an increase in the total exchange capacity of the remaining exposed surface. This is demonstrated clearly by the data in table 3 for plots 2 and 9, on which excessive erosion occurred, and by plot 10, which was artificially eroded by removing 7 inches of surface soil in 1930. By calculating the soil loss in tons per acre on the acre-inch basis and using this figure to determine the amount of surface soil removed, it is evident that progressive amounts of the subsoil became mixed with the surface soil each year in which it was tilled to a constant depth for the following crop. Physical analyses of a typical profile from these plots show that the clay content increased with depth (6). As the progressive mixing of the surface and subsoil continued, the total exchange capacity increased, since clay content and total exchange capacity are closely associated.

Although plot 1 cannot be compared directly with the other plots, because it is twice their length, the data in table 3 show that as erosion increased, the exchange capacity decreased in the upper half and increased in the lower half of the plot. It is known that, as the length of slope increases for this type of soil (12),<sup>6</sup> the amount of soil lost increases. This increase was greater on the lower half of the plot, where more of the subsoil was eroded. The upper half of the plot shows the effect that organic matter has on total exchange capacity. As the organic matter decreased, the exchange capacity also decreased. With but a small soil loss, and with the organic matter remaining constant (plots 3, 4, 5) or increasing (plots 6, 7, 8), the total exchange capacity remained at approximately the same level for the 7-year period.

#### EXCHANGEABLE CALCIUM AND MAGNESIUM

An examination of the data for plots 1, 2, 9, and 10 in table 3 shows that a general parallelism exists between the exchangeable bases and the clay content. In these plots erosion has removed various amounts of surface soil, and this should, according to determinations of the profile reported elsewhere (6), result in the incorporation of increasing amounts of clay from the subsoil into the surface soil. Since these plots (1 and 2, continuous corn; 9 and 10, fallow) received only small increments of organic matter or none, the effect of organic matter on the exchangeable bases is minimized. This is clearly shown by plot 2, where, as the percentage of organic matter decreased, an increase in clay content counteracted the decrease to maintain an approximately constant level of exchangeable bases.

The effect of organic matter on exchangeable calcium also is demonstrated. Where the amount of soil eroded was small, but an increase (plot 8) or decrease (plots 3, 4, 5) in organic matter had occurred for the period, there were corresponding increases or decreases in exchangeable calcium. As was to be expected for plots 6 and 7, fertilizer and limestone treatments materially increased the milliequivalents of exchangeable calcium.

<sup>6</sup> See footnote 4.

## PH VALUES

There was no direct relationship between pH and rate of soil loss. There appeared to be no relationship between pH and exchange capacity, or exchangeable calcium and magnesium; McGeorge (5) previously reported a similar lack of relationship.

## ORGANIC MATTER AND NITROGEN

There appears to be a relationship between surface-soil removal and the percentage of organic matter and total nitrogen content in the remaining soil (table 3). In the continuous bluegrass plot there was a progressive increase of organic matter and nitrogen and a negligible loss of soil, in contrast to a progressive decrease of organic matter and nitrogen with high soil losses in the fallow and continuous corn plots.

Comparison of the nitrogen content of the continuous bluegrass plot with that of the rotation plots (plots 3, 4, 5, 6) shows that the latter did not reach the nitrogen level attained by the bluegrass plot. This observation agrees with that of Jenny (4) on the effect of farming operations on the nitrogen content of the soil.

When the data for the untreated rotation plots 3, 4, and 5 are averaged by years it is found that this rotation did not maintain the original level of organic matter and nitrogen. Some idea of the effect that soil treatment may have can be obtained by comparing plot 6 (treated) with plot 5 (untreated). Although the original level of nitrogen and organic matter was lower on plot 6, the values in table 3 show that on this plot (rotation with soil treatment) the organic matter and nitrogen content were maintained, while on plot 5 (rotation without soil treatment) both decreased slightly. The treated rotation not only maintained the organic matter and nitrogen, but operated also to decrease the soil loss.

## DISCUSSION AND CONCLUSIONS

Soil surveyors know that it is often difficult to ascertain how much of the original surface soil has been removed by erosion. This is true especially where the surface soil has been mixed with the underlying subsoil by plowing. With the plots studied, all having had different rates of losses, depending upon the crop and cultural treatment, some understanding of the relative position of each plot with regard to native profile conditions may be obtained by comparison with the 0-to-13-inch standard profile.

In order to compare the data from other plots with the profile of plot 8 (continuous bluegrass), it was necessary to plot the magnitude of the soil property under consideration against soil loss. Since soil loss from plot 8 was negligible, and since the annual composite samples were taken from the top 7 inches, the average of the top seven 1-inch horizons of this plot was taken as zero soil loss. This procedure is logical, since originally all plots had the same soil depth, except plot 10 which was desurfaced.

Two lines are plotted in figures 3 and 4 from the data for plot 8. Both lines are made of data secured from the analysis of samples collected in 1939, but corrected for each 1-inch horizon, to such extent as the average of the seven 1-inch horizons in 1939 varied from the value of the single 7-inch sample in 1931. No samplings other than those of 7-inch horizons previously had been made. While this



method of correction may be open to criticism, it was taken as the best at hand for comparative purposes.

The broken line in figures 3 and 4 results when it is assumed that successive 1-inch layers of soil are lost from the surface, which would

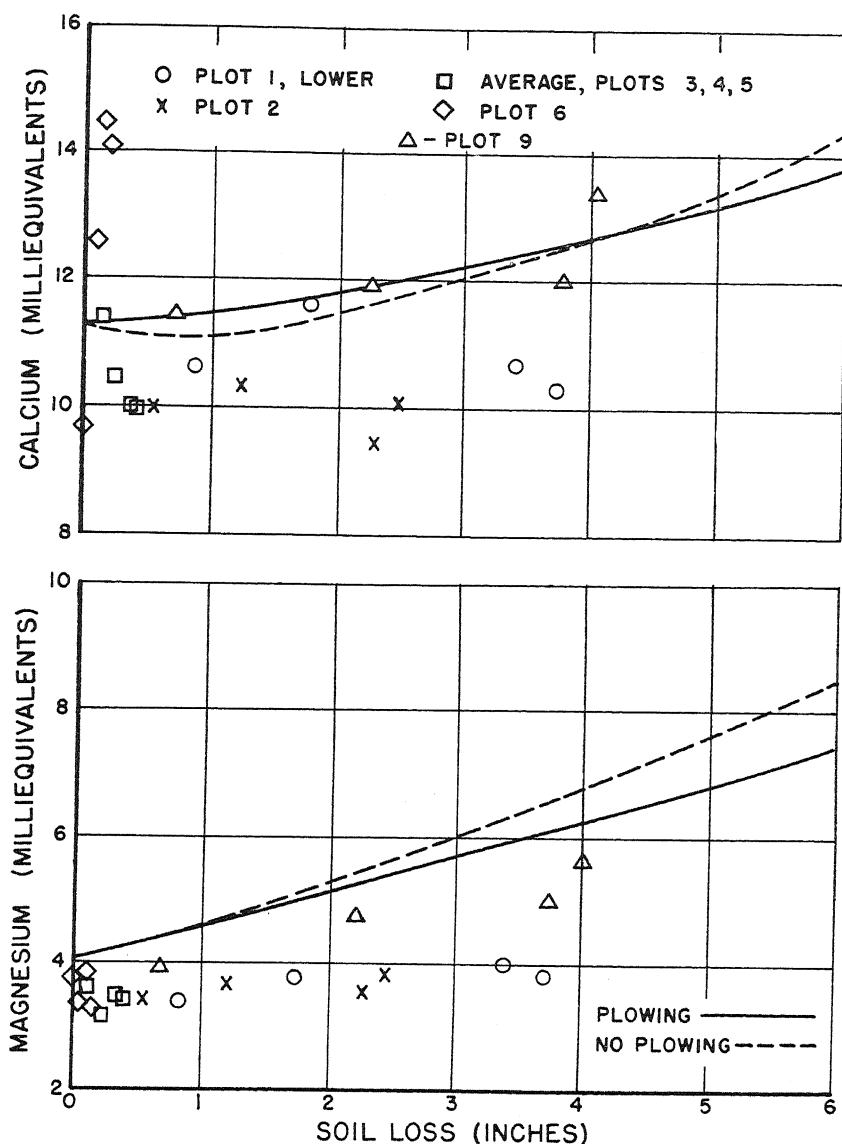


FIGURE 3.—Changes in exchangeable calcium and magnesium in the surface soil with erosion, without plowing and with plowing to a depth of 7 inches.

be the case without plowing. This loss amounts to a running average of 7 inches, using the data in table 2. The solid line represents the results obtained when plowing to a depth of 7 inches is assumed each time 1 inch of soil is lost. By the use of this last method an attempt

is made to consider the mixing effects resulting from tilling the soil for cultivated crops.

No attempt was made to compare directly the plots in series 1 with selected 1-inch horizons of the continuous bluegrass plot; emphasis

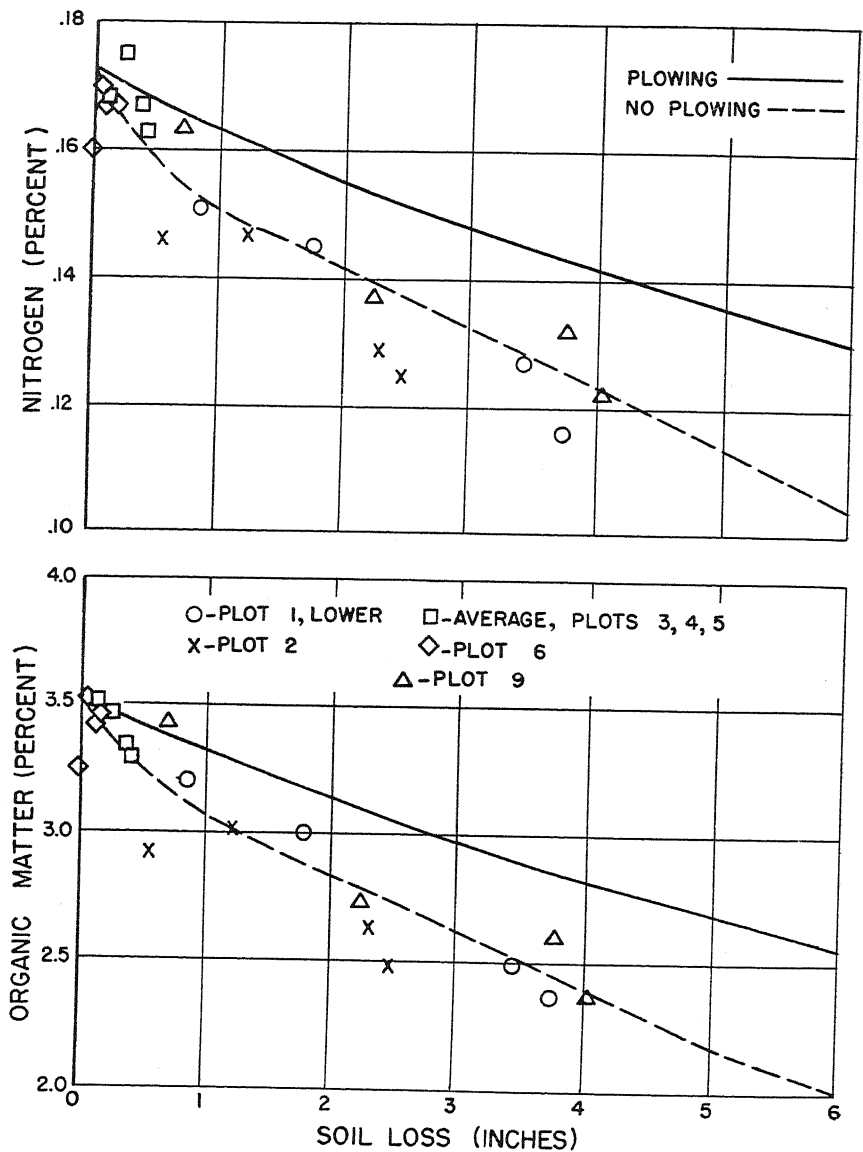


FIGURE 4.—Changes in organic matter and nitrogen in the surface soil with erosion, without plowing and with plowing to a depth of 7 inches.

was placed rather on observing the trend of the values in these plots and comparing them with the values obtained for the continuous bluegrass plot.

Figure 3 illustrates the fact that when 1 inch of topsoil is removed

by erosion, it does not necessarily follow that the exchangeable calcium and exchangeable magnesium in the remaining soil profile will conform to that of the respective horizons contained under native profile conditions (0- to 13-inch profile). However, the plotted results show that with progressive accumulative erosion losses, which means that the profile will be truncated more each year, the soil properties tend to follow the design of the values obtained for the native profile. For the Shelby loam, the results show that as depth increases, the total exchange capacity and exchangeable bases increase. Slater and Byers (10) reported similar results for the exchangeable bases of the soil colloids by definite horizon depths for this soil profile.

From figure 4 it would seem that the organic-matter and nitrogen content change at very nearly the rate indicated by plot 8, when no plowing is assumed (broken line). But since all plots except 8 were tilled—plots 1, 2, and 9 annually and 3, 4, 5, and 6 every 3 years—they might be expected to follow the solid line (plowing). The fact that decreases in organic matter and nitrogen are greater than would be expected may be explained by the fact that the line plotted for plot 8 takes into account only the losses subsequent to erosion, and not those that accompany crop production and oxidation brought about by plowing.

Slater and Carleton (11) reported earlier on the loss of soil organic matter in this region. They found that organic-matter content dropped 0.002 percent on both Shelby and Marshall soils for each ton of soil lost by erosion. This value was calculated by assuming a linear regression, and they point out that "the line in reality must be a curve since the organic-matter content of the soil cannot be reduced below zero, while erosion can continue indefinitely."

The results of this study indicate that the rate of loss of organic matter and nitrogen decreases, which also gives a curved line. Albrecht (1) points out that—

this high rate of depletion will not continue. As is true for all biochemical processes, the early rate of consumption is rapid, which gives a sudden decrease.  
\* \* \* Long continued experiments, accompanied by soil analyses, prove that the organic-matter content of a soil will reach a fixed level characteristic of the surrounding climatic conditions.

To climatic conditions may be added cropping practices. The bluegrass and alfalfa plots are somewhat above the natural equilibrium in organic-matter content (3.54 percent) for this section of Missouri (1); a fertilized rotation of corn, wheat, clover with timothy is approximately in equilibrium; and the same rotation unfertilized is slightly below. It would seem that the plots in corn and fallow have passed the period of sharp decline, and now are decreasing in organic-matter content at a slower rate.

It is of interest to note that, for a 3-year rotation of corn, wheat, and clover-timothy with fertilizer and lime treatment, the fertility of the soil was not only maintained but the yields were increased. Yield-data records for the period show increases of 45 percent for clover-timothy, 69 percent for wheat, but no significant difference for corn as compared with the nontreated plot. Observations suggest that a drought which occurred at a critical period when the corn was maturing had a greater effect on yield than any other single factor.

The results obtained do not indicate that any one soil property can be used as a criterion to evaluate the amount of soil loss, or more

specifically, the amount of surface soil remaining. However, by considering several soil properties it would seem that an evaluation can be made. Of the soil properties studied, organic-matter and nitrogen content appear to indicate most nearly the degree of truncation of soil profiles by erosion.

Values obtained from a study of the 0- to 13-inch horizon indicate that, if the rate of erosion is small, the amounts of subsoil brought to the surface by successive truncations of the profile by erosion will be of benefit to the surface horizon. Exchangeable calcium and magnesium lost through leaching, crop removal, and erosion would be replenished partly or wholly. Nitrogen and organic-matter deficiencies would be corrected by the combined activities of micro-organisms and legumes, since conditions for their growth and development would be favorable. The use of lime and phosphate would still be desirable for maximum yields.

From the recent work of Graham (3) it would seem that the average soil loss of 0.025 acre-inch per year from plot 6 would not be so great that the primary minerals incorporated from the subsoil with the surface soil would lower the soil-fertility level. Mineralogical analyses show that this soil contains appreciable amounts of calcium-bearing minerals.<sup>7</sup> Middleton et al. (6) found that the percentage of CaO increased with an increase in profile depth.

Unpublished data by Woodruff and Simerly<sup>8</sup> show that under field conditions sand and silt may accumulate as a result of the clay fraction being carried off in suspension by the run-off water. Data reported by Middleton et al. (7) for the years 1931 and 1932 for these plots are in agreement with the foregoing conclusions; there is a marked increase in clay content in the soil eroded from the plots as compared with the plot composites.

For the plots fallowed and for those in cultivated crops the mechanical composition of the eroded material is similar to that of the soil remaining. For the uncultivated crops the eroded material is finer in texture than is the surface 7 inches of soil. Therefore, the texture of the soil should not change materially from the original by additions of clay from the subsoil.

The data in table 1 show that a rotation not only keeps the soil loss at a practical minimum, but also greatly reduces surface run-off, as compared with the continuous corn crop. Unpublished results of a study of the relation between precipitation and surface run-off for these plots for a 9-year period (1931-39) show that bluegrass was three times as effective and a rotation of corn, wheat, clover-timothy twice as effective as continuous corn in preventing run-off.<sup>9</sup>

As the cultural operations were with the slope, it appears logical to conclude that if these operations were carried out on the contour, the soil and water losses would be reduced further. Data from experimental area 5-N, which is similar to the fertilized rotation plot in soil type, slope, and treatment, except that it represents a field actually terraced and contour cultivated, show that the soil loss for a 6-year period (1932-37) was 1.02 tons per acre per year. At this

<sup>7</sup> Unpublished soil mineralogical data, by J. R. Johnson. Iowa Agr. Expt. Sta. 1939.

<sup>8</sup> WOODRUFF, C. M., and SIMERLY, M. E. THE SIZE DISTRIBUTION OF MATERIALS ERODED FROM DIFFERENT SLOPES. Unpublished data. Mo. Agr. Expt. Sta. 1940.

<sup>9</sup> SMITH, D. D., and SWANSON, C. L. W. A PRELIMINARY STUDY OF THE RELATIONSHIP BETWEEN PRECIPITATION AND SURFACE RUN-OFF. Unpublished data. U. S. Soil Conserv. Serv. Res., Conserv. Expt. Sta., Columbus, Mo. 1940.



rate of soil loss, 980 years would be required to remove the surface 7 inches.

These data also suggest that the application of conservation measures on a practical field basis to reduce the deleterious effects of erosion would not only reduce to a practical minimum soil and water losses, but would also maintain the fertility of the soil.

It appears possible that soil of types similar to Shelby loam can be rejuvenated and conserved for years to come. The data obtained seem to suggest that there is some benefit to be gained by the addition of small increments of subsoil to the surface soil, if at a slow enough rate and when combined with the organic matter introduced by proper cropping. This result may be achieved by employing judicious soil-, crop-, and conservation-management practices.

#### SUMMARY

Data are presented showing the results of analyses for total exchange capacity, exchangeable calcium, exchangeable magnesium, organic-matter content, nitrogen content, and hydrogen-ion concentration of Shelby loam from plots planted to different crops and maintained under different cultural conditions at the Soil Conservation Experiment Station, Bethany, Mo. Results of the same determinations on 1-inch horizons of a 0 to 13-inch profile continuously in bluegrass also are reported for comparative purposes.

According to the data for the profiles by 1-inch horizons, the exchange capacity, the exchangeable bases, and the base saturation increase whereas the organic matter and nitrogen content decrease with increasing depth.

Nitrogen and organic matter appear to decrease at a decreasing rate as erosion progresses. In general a decrease in organic matter and nitrogen occurred with increased soil loss. This decrease occurred at a rate below that to be expected from the profile study.

The base exchange properties follow the trend of the values for the 0 to 13-inch profile of the bluegrass plot. The effect of organic matter on these properties is pointed out.

It was found that the fertility of the soil was maintained by a 3-year rotation of corn, wheat, clover-timothy with fertilizer and lime treatments. The rate of soil loss was small enough so that the deleterious effects were counteracted by the incorporation of small increments of the subsoil. The clay added from the subsoil was removed in the surface run-off in such amounts as not to alter materially the texture of the soil. Additions of subsoil increased the amounts of exchangeable calcium and magnesium.

No relation was observed between the concentration of hydrogen-ions in the soil and soil loss.

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# FACTORS AFFECTING DISTRIBUTION AND SEVERITY OF BLACK ROOT ROT OF APPLE TREES<sup>1</sup>

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## INTRODUCTION

Certain aspects of the geographic, ecologic, and host-parasite factors that appear to affect the distribution and severity of black root rot of apple trees, caused by *Xylaria mali* Fromme, and experiments dealing with some physiological studies of the pathogen are reported herein. Since the distribution of this root rot is restricted, a study of its environmental limitations, together with inoculation experiments, should throw some light on the conditions favoring or hindering infection.

## DISTRIBUTION AND SEVERITY OF BLACK ROOT ROT

Black root rot is distributed from Pennsylvania to Georgia and westward to Arkansas. Serious losses have been confined to the central and southern parts of the range, and the losses in Pennsylvania and Maryland have been unimportant. The greatest losses have occurred in the fruit-growing sections of Virginia, West Virginia, and the States to the south and also in the Ozark region. Although the root rot has been observed in the Coastal Plain, which is not an extensive fruit-growing section, it has not caused severe losses there. The general distribution of the disease is probably influenced directly or indirectly by temperature. As will later be shown (p. 302), the optimum temperature, about 25° C., for the strain of the pathogen studied is fairly high for a soil-inhabiting organism. Temperature may be a factor in the frequent occurrence of the disease in Virginia and other Southern States and in its infrequent occurrence in Pennsylvania.

High temperature may influence susceptibility by favoring the pathogen or by adversely affecting host resistance.

Even within the range of its most frequent occurrence there is considerable irregularity in the distribution of the disease. No adequate explanation for this can be given at this time. Among the possible contributing factors may be genetic differences in the parasite. A study of the pathogenicity of different isolates, reported elsewhere in this paper (p. 303), gives some ground for this conclusion. Probably the most important factor, however, that affects the local distribution of the disease is the edaphic environment of the host.

Possibly a deficiency of moisture is concerned in the local abundance of the disease. Many instances have been observed in which the disease was much more prevalent on a knoll where moisture and fertility were presumably less favorable to tree growth than in the rest of the orchard. However, consideration should be given the

<sup>1</sup> Received for publication April 22, 1942.

following correspondence from F. J. Schneiderhan, who, with F. D. Fromme, spent several years in intensive work on this disease in the Shenandoah Valley apple section of Virginia:

I have found more root rot in orchards growing in rich orchard soil than in poor soil. Most of Fromme's and my observations were made in the better orchards in Virginia and West Virginia. I admit that we found considerable mortality on Ben Davis orchards after the 1930 drought, but in most of these cases root rot was associated with blister canker and I do not know which was the major factor. \* \* \*

Some of the severest infections found near Winchester were at the lower part of orchard slopes where moisture content and soil nutrients were highest. I have always believed that the availability of fungus inoculum was the important factor in the localization of infection within an orchard.

The distribution and the severity of the disease are probably dependent on a number of interrelated and concomitant conditions.

### INFECTION WITH *XYLARIA MALI*

#### MATERIALS AND METHODS

Prior to 1936 inoculation work was conducted at Arlington Experiment Farm, Arlington, Va., on nursery trees 2 to 5 years old. In 1936 and later inoculations were made at the United States Horticultural Station, Beltsville, Md. Natural infection by *Xylaria mali* has not been found in these locations, but experience has shown that apple trees growing in these localities may be fairly readily infected by inoculation with pure cultures of the fungus. Unless otherwise stated, the material and methods used were those described below.

Roots of apple (*Malus pumila* Mill.) trees were inoculated in situ by removing enough soil to expose root tissue; against this was laid a piece of apple twig, about 2 inches long, on which a profuse growth of the fungus had been produced in artificial culture. The soil was replaced after inoculation and not disturbed until records were taken, usually at the end of the growing season. In the inoculations made in 1932 and 1933, a wound was made into the cambium. All subsequent inoculations were made by applying the inoculum to a shallow wound made by scraping with a trowel. Unless otherwise stated, the inoculum was a highly pathogenic clone (No. 57) isolated from an infected apple root from Bluemont, Va., and the field inoculations were made in July.

Where inoculations were made on trees that had been dug, the inoculum was held by a rubber band against an open incision in the root and after inoculation the roots were wrapped in paper and stored in moist peat.

Since infection did not occur on uninoculated trees or even on uninoculated portions of inoculated roots, it was not considered necessary to leave part of each plot uninoculated as a check on infection.

#### EFFECT OF KIND AND QUALITY OF INOCULUM

On February 2, 1934, inoculations were made to determine the effect of the character and quantity of inoculum used. One-year-old apple seedlings were sorted into uniform lots of 10 trees each, 4 incisions were made in each tree, and each wound was inoculated with 1 of 3 types of inoculum. The inoculum was (1) a mass of mycelium and agar from an agar culture, (2) a small chip from an



inoculated heat-sterilized apple twig on which the fungus had been allowed to grow for 2 months, or (3) a piece of twig 2 inches long prepared as in (2). After inoculation, the seedlings were packed in moist peat and held in a cool room (15° to 20° C.). The results of this experiment are given in table 1.

TABLE 1.—Influence of type of inoculum on infection of apple seedlings inoculated with *Xylaria mali*, Feb. 2, 1934

[10 trees inoculated at 4 incisions each]

Type of inoculum	Results on Apr. 27, 1934	
	Incisions infected	Average length of lesions
	Percent	Mm.
Mycelium from an agar culture.....	0	.....
Small chip of inoculated wood.....	0	.....
Small inoculated twig.....	60	15.6

Under the conditions of this experiment, inoculations with agar-culture inoculum and with a small chip of inoculated wood were not successful; apparently the fungus died before infection took place. A fair percentage of infection resulted when the inoculum was a fairly large piece of infected twig.

#### EFFECT OF WOUNDING

Field observations indicate that *Xylaria mali* readily attacks apparently uninjured roots, and therefore wounding seems to be unnecessary for natural infection. To determine the influence of wounding on dug trees, inoculations were made on February 2, 1934 on 1-year-old apple seedlings stored in moist peat in a cool room (temperature 15° to 20° C.). The seedlings were divided into 2 comparable lots of 20 trees each. An incision (flap cut) was made in each seedling of 1 lot and a twig culture of the fungus was fastened to it by a rubber band. The other lot was inoculated in the same way but without wounding. The results of the experiment appear in table 2.

This experiment indicates that wounding is not necessary for infection of stored trees by *Xylaria mali*. The fact that fewer infections and smaller lesions occurred on the wounded trees may have been due to the wound callus, which may be less susceptible to attack than normal root tissue.

TABLE 2.—Effect of wounding upon infection of apple seedlings inoculated with *Xylaria mali*, Feb. 21, 1934

[20 trees in each lot]

Host treatment	Results on Apr. 27, 1934	
	Trees infected	Average length of lesion
	Percent	Mm.
Inoculum inserted in a flap-cut wound.....	50	16
Inoculum applied without wounding.....	80	20

## EFFECT OF TEMPERATURE

Two Petri plates of string-bean agar inoculated with *Xylaria mali* (isolate 57) on November 23, 1937, and held at each of the indicated temperatures for 14 days contained mats of the average sizes shown in the following tabulation:

Temperature (° C.)	Diameter of mat after 14 days Mm.
40.....	0
35.....	0
30.....	65
25.....	75
20.....	55
15.....	17.6
10.....	9.3
5.....	0
0.....	0

The results indicate that, although this isolate will grow in culture at a temperature as low as 10° C., its optimum is near 25°.

In order to learn the effect of temperature on root infection, the roots of yearling apple seedlings were inoculated at two places each with isolate 57 by attaching pieces of twig inoculum without wounding. The seedlings were then wrapped in moist paper, and 12 were put in each of two 8-liter jars. One jar was placed in a controlled-temperature room held at 15.5° C., and the other was held in the laboratory at about 21°. This experiment, run in 1934, was repeated in 1935, and a third lot of seedlings was held at outside temperatures (−3° to 18° C.). The results of these experiments are reported in table 3.

TABLE 3.—Effect of temperature on infection of yearling apple seedlings by *Xylaria mali*

[12 seedlings in each lot]

Temperature (° C.)	Results of Mar. 17, 1934, inoculations (after 2 months)		Results of Mar. 16, 1935, inoculations (after 1½ months)	
	Trees infected	Average length of lesions	Trees infected	Average length of lesions
	Percent	Mm.	Percent	Mm.
−3 to 18 (outside).....	.....	.....	0	.....
15.5 (controlled).....	31	16	83	11
21 (room).....	84	22	96	21

The data show that *Xylaria mali* will grow on culture media at a temperature as low as 10° C. and that it will infect stored apple seedlings at a temperature as low as 15.5°, which is much lower than that of the top layers of soil during much of the summer where this disease is prevalent. In 1935 the seedlings were in poor condition at the conclusion of the experiment, but they were still alive. Attention should be called to the fact, however, that conditions affecting resistance in trees that have been dug and stored may be different from those in undisturbed trees.

## RELATIVE PATHOGENICITY OF DIFFERENT ISOLATES

The relative pathogenicity of different isolates from naturally diseased apple roots was determined by growing them on sterilized apple twigs, which in turn were used to inoculate the roots of stored yearling apple seedlings. In 1933 the seedlings were divided into uniform lots of 10, and in 1934 into lots of 8. Each lot was wrapped in moist paper, packed in moist peat, and placed in a cool room with a temperature range of 15° to 20° C. until the final record, as shown in table 4, was made.

TABLE 4.—Relative pathogenicity of different isolates of *Xylaria mali* on yearling apple seedlings

[10 seedlings inoculated with each isolate on Nov. 30, 1933, and 8 with each on Apr. 6, 1934. Final record made on Jan. 10 and May 18, 1934, respectively]

Year of inoculation and isolate No.	Source of inoculum	Trees infected	Average length of lesion	Year of inoculation and isolate No.	Source of inoculum	Trees infected	Average length of lesion
		Percent	Mm.			Percent	Mm.
1933							
3	Markham, Va.	60	22.1	127	Front Royal, Va.	100	14
28	Hillsboro, Va.	30	19.7	142	Leesburg, Va.	100	14
30	Winchester, Va.	0		149	do	87.5	18
56	Linden, Va.	0		152	do	87.5	14.7
57	Bluemont, Va.	100	42.5	158	do	75	11.7
58	do	50	26.4	161	do	87.5	9.7
59	do	70	39.1	164	do	100	14.2
61	Purcellville, Va.	30	19.3	168	do	87.5	13.3
63	Leesburg, Va.	30	27.3	172	do	100	13.9
				182	do	50	7.7
1934				197	do	0	
29	Charles Town, W. Va.	0		198	do	100	20.2
103	Bluemont, Va.	0		199	do	75	17
117	Winchester, Va.	50	8.5	200	do	100	18.5
126	Martinsburg, W. Va.	37.5	6.6				

Table 4 shows great variation in the pathogenicity of 27 isolates from 10 different localities in Virginia and West Virginia. Infection ranged from 0 to 100 percent, and average length of lesion from 6.6 to 42.5 mm. The pathogenicity of the fungus on stored trees might be different from that on trees growing in the field. This experiment, however, suggests that some of the irregular distribution and severity of the disease may be associated with variation in pathogenicity of strains of the organism as well as with edaphic conditions. Fromme (2)<sup>2</sup> also found differences in the pathogenicity of isolates.

## COMPARATIVE SUSCEPTIBILITY OF APPLE, PEAR, PLUM, CHERRY, AND PEACH TREES

During the greater part of 1933 and 1934 monthly inoculations were made on 3- to 5-year-old seedling trees of apple, pear, plum, cherry, and peach. In the tests made in June and July, the period of greatest susceptibility, at least 20 trees of each species were used at each inoculation period, but at some of the other periods only 5 to 10 trees were inoculated. During a period of high susceptibility 40 to 80 percent of the apple trees and 5 to 10 percent of the pear trees became infected. Many of the lesions on the apple trees were typical deep lesions, but on the pear trees they were shallow and soon healed over.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 311.

On young mazzard and mahaleb cherry seedling trees infection would sometimes advance as much as 10 to 20 mm., and by the next year the lesions would have healed over. Fromme (2) reported infection from inoculation on apple, pear, cherry, elm (*Ulmus americana*), honeylocust (*Gleditsia triacanthos*), and Norway maple (*Acer platanoides*). Schneiderhan (5), in a popular report, stated that oak, maple, hackberry, grape, sassafras, and ash were infected by inoculation. Neither of these workers, however, stated whether the lesions continued to enlarge or whether they soon healed over. However, Schneiderhan did state in recent correspondence:

\* \* \* On the Dutrow orchard near Charles Town, W. Va., Fromme and I found several cherry trees that were dead from *Xylaria mali* after having been planted where an apple tree had been previously killed by the fungus. I strongly suspect that pear trees are also susceptible.

Several hundred inoculations on peach and myrobalan plum seedling trees all gave negative results. In many field examinations the writer has never found *Xylaria* attacking the roots of cherry, peach, or plum trees growing near or interplanted with diseased apple trees. In one instance in the Shenandoah Valley of Virginia, peach trees were planted where apple trees known to be infected with *Xylaria* had been removed, and after 4 years no evidence of black root rot was found on them.

On the other hand, J. O. Andes, of the Tennessee Agricultural Experiment Station, mentioned, in conversation with the writer, that *Xylaria* had effectively attacked a peach tree growing where an apple tree infected with *Xylaria* had been removed. The experiments herein reported and the field observations of the writer seem to bear out the belief that peach, plum, cherry, and possibly pear trees are sufficiently resistant to be grown successfully on land from which a diseased apple orchard has been removed.

#### SUSCEPTIBILITY OF SEEDLING CLONES AND OF SEEDLING AND OWN-ROOTED APPLE VARIETIES

Experimental plots of trees growing at the United States Horticultural Station, Beltsville, Md., used for testing possible differences in root susceptibility to infection with *Xylaria* were of three types. Part were own-rooted apple varieties, part were clonal lines of seedlings that had shown merit for understock use, and part were seedlings of standard varieties. The own-rooted varieties had been started with nurse roots and had grown for two seasons; the clones of seedling origin had grown for two seasons from root cuttings; and the variety seedlings were 1 year old when obtained for this experiment. The trees were set 2 feet apart in nursery rows 5 feet apart. The plots of own-rooted varieties contained from 9 to 20 trees each. The variety seedling and clone plots contained from 10 to 20 trees, usually 20.

The trees were allowed to grow undisturbed till midsummer of the second year, at which time they were inoculated with twig cultures of *Xylaria* isolate 57 applied to the main root without deep wounding. Only one inoculation was made on each tree in a single year. The results of inoculation were recorded at the end of the growing season, or after October 15. The lesions that developed usually healed over during the following season. By inoculating opposite sides of the roots in successive years, repeated inoculations have been



made. The own-rooted seedling varieties were inoculated for 4 different years and the others for 3 years.

The results of this work are reported in tables 5 to 7, wherein lesions are divided into two main groups—shallow infections of the cortex only and deep infections, which penetrate into the cambium. Cortical infections are the result of host-parasite reactions of such a nature that the fungus attack is checked before the cambium is killed, and these shallow lesions heal much more rapidly than deeper lesions. A high ratio of cortical to deep infections may be an important indication of host resistance.

The sizes of the deep lesions, as given in the last column of tables 5 to 7, were obtained in the following manner: For each lesion, the length was multiplied by the breadth to determine the approximate area. The square root of the resultant area was then derived, giving

TABLE 5.—*Infection and size of deep lesions on young own-rooted apple trees inoculated with Xylaria mali*

[Varietal means from approximately 36 to 80 readings on 9 to 20 trees of each variety; each tree was inoculated in 1936, 1937, 1938, and 1939]

#### EXPERIMENTAL DATA

Variety	Mean of varieties			
	Total infection	Cortical infection only	Deep infection <sup>1</sup>	Size of deep lesions
	Percent	Percent	Percent	Mm. <sup>2</sup>
Arkansas (Black Twig).....	90.0	50.0	40.0	26.2
Ben Davis.....	75.0	25.0	50.0	21.9
Delicious.....	91.3	32.4	58.9	32.7
Fallawater.....	88.9	36.1	52.8	21.0
Golden Delicious.....	64.3	42.8	21.5	15.6
King David.....	72.4	43.3	39.1	23.1
McIntosh.....	78.2	35.2	43.0	20.2
Northern Spy.....	65.2	23.0	42.2	19.2
Opalescent.....	60.8	23.6	31.2	28.7
Perkins.....	75.0	41.1	33.9	18.9
Red Astrachan.....	70.8	43.5	27.3	14.6
Rome Beauty.....	50.0	20.8	29.2	13.3
Smith Cider.....	63.5	28.2	35.3	22.7
Stayman Winesap.....	91.0	17.5	72.5	34.8
Summer Rambo.....	89.6	22.9	66.7	27.8
Tolman Sweet.....	76.1	21.7	54.4	27.9
Wealthy.....	86.7	23.4	63.3	24.4
Yellow Transparent.....	68.0	31.3	36.7	20.9
York Imperial.....	78.6	54.3	24.3	16.1
All varieties: <sup>3</sup>				
1936.....	47.7	-----	22.9	11.1
1937.....	76.1	-----	25.7	16.8
1938.....	82.3	-----	70.0	27.0
1939.....	95.7	-----	54.5	24.0

#### ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square <sup>3</sup>		
		Total infection	Deep infection	Size of deep lesion
Variety.....	18	560.53	883.15*	197.04
Years.....	3	7,735.23**	9,871.94**	1,082.01
Variety × years.....	54	401.24	430.84	510.15

<sup>1</sup> Twice the standard error of the difference for deep infection is 29.4. A difference between any 2 values, therefore, that amounts to as much as 29.4 is considered statistically significant (19:1).

<sup>2</sup> In yearly means twice the standard error of the difference for percent total infection is 13.0, for percent deep infection it is 13.5, and for size of deep lesion it is 14.8. Differences greater than these values, therefore, are considered statistically significant (19:1).

<sup>3</sup> \* = Significant (19:1); \*\* = highly significant (99:1).

a linear value equal to one side of a square having an area proportionate to that of the lesion. The values recorded in the table are averages of such linear functions for all lesions in each designated plot. Since the lesions were irregular in outline and the ratio of surface killing to cambium killing might not be the same, it was difficult to obtain a high degree of accuracy in such measurements. The sizes of deep lesions, therefore, as given in the tables, represent approximate rather than absolute values.

TABLE 6.—*Infection and size of deep lesions on young apple seedling clones inoculated with Xylaria mali*

[Clone means from approximately 30 to 60 readings on 10 to 20 trees of each variety; each tree was inoculated in 1936, 1937, and 1938]

# EXPERIMENTAL DATA

Seedling clone No.	Mean of clones			
	Total infection	Cortical infection only	Deep infection <sup>1</sup>	Size of deep lesions
	Percent	Percent	Percent <sup>1</sup>	Mm.
227 <sup>2</sup>	43.5	4.2	39.3	28.1
309	76.9	7.7	69.2	29.3
316	59.4	29.7	29.7	20.1
317	50.8	36.5	14.3	37.4
323 <sup>2</sup>	87.5	19.1	68.5	50.8
329	61.1	36.1	25.0	28.8
1223	48.7	20.5	28.2	17.4
1224	77.7	38.8	38.9	24.2
1225 <sup>2</sup>	78.4	24.8	53.6	31.1
1226	29.2	16.3	12.9	15.5
1227	54.5	40.6	13.9	15.2
1232	50.9	28.7	22.2	23.8
1241	25.0	12.5	12.5	27.2
1249	37.8	17.8	20.0	30.3
1251	48.9	35.6	13.3	23.7
1256	36.4	18.2	18.2	19.3
1258 <sup>2</sup>	30.0	20.0	10.0	12.8
1263	28.2	12.5	15.7	25.7
1267	47.6	26.8	20.8	31.1
1269 <sup>2</sup>	40.9	20.7	18.2	33.9
1271	40.7	22.2	18.5	20.8
1272	26.6	16.6	10.0	28.3
1273	45.7	22.4	23.3	21.6
1283	72.9	36.3	36.6	27.4
1291	58.1	29.3	20.8	12.3
1297	62.4	26.1	36.3	19.2
1299	51.0	16.5	34.5	24.4
1300	52.7	34.9	17.8	23.9
1302	65.9	20.9	45.0	38.1
1303	25.9	18.5	7.4	21.2
All clones: <sup>3</sup>				
1936	26.90		16.67	9.46
1937	47.58		1.25	2.09
1938	69.32		51.19	23.46

# ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square <sup>4</sup>		
		Total infection	Deep infection	Size of lesion
Variety	23	643.73	301.96	79.26
Years	2	10,795.18**	12,290.12**	2,828.16**
Variety X years	46	668.57	454.55	102.45

<sup>1</sup> Twice the standard error of the difference for deep infection is 35.0. A difference between any 2 values, therefore, that amounts to as much as 35.0 is considered statistically significant (19:1).

<sup>2</sup> Data lacking for 1 year; not included in the statistical summary.

<sup>3</sup> In yearly means twice the standard error of the difference for percent total infection is 14.9, for percent deep infection it is 12.3, and for size of lesion it is 5.8. Differences greater than these values, therefore, are considered statistically significant (19:1).

<sup>4</sup> \*\*\*= Highly significant (99:1).

TABLE 7.—*Infection and size of deep lesions on young apple seedlings inoculated with Xylaria mali*

[Varietal means from approximately 30 to 60 readings on 10 to 20 seedlings of each variety; each seedling was inoculated in 1936, 1937, and 1938]

## EXPERIMENTAL DATA

Variety	Mean of varieties			
	Total infection	Cortical infection only	Deep infection <sup>1</sup>	Size of deep lesions
	Percent	Percent	Percent	Mm.
Ben Davis.....	56.4	8.1	48.3	26.8
Delicious.....	48.1	23.7	24.4	21.8
Fameuse.....	53.1	14.4	38.7	21.4
Jonathan.....	58.7	20.5	38.2	19.6
McIntosh.....	60.3	21.2	39.1	29.6
Northern Spy.....	47.0	20.1	26.9	21.5
Rome Beauty.....	44.6	14.4	30.2	20.6
Red Rome Beauty.....	59.2	11.4	47.8	30.9
Wealthy.....	44.7	20.8	23.9	34.2
Commercial (mixed).....	43.7	6.0	37.7	32.7
All varieties; <sup>2</sup>				
1936.....	45.63		31.52	26.67
1937.....	31.95		3.73	6.99
1938.....	77.27		71.36	27.20

## ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square <sup>3</sup>		
		Total infection	Deep infection	Size of deep lesion
Variety.....	9	133.68	236.57	57.01
Years.....	2	5,403.55**	11,555.54**	1,326.71**
Variety X years.....	18	440.76	507.01	124.97

<sup>1</sup> Twice the standard error of the differences for deep infection is 36.8. A difference between any 2 values, therefore, that amounts to as much as 36.8 is considered statistically significant (19:1).<sup>2</sup> In yearly means twice the standard error of the difference for percent total infection is 18.8, for percent deep infection, it is 20.1, and for size of lesion it is 10.0. Differences greater than these values, therefore, are considered statistically significant (19:1).<sup>3</sup>\*\* = Highly significant odds (99:1).

In general, resistance was greater at the first inoculation, and in some cases at the second, than at later ones. Many of the plots showed no deep infections at all from the first inoculation. An entirely adequate explanation cannot now be given for this fact. Evidence points to the conclusion, however, that resistance to infection is more probably associated with host conditions favorable for infection than with environmental conditions unfavorable for the growth of the pathogen. The work in general indicates that young trees are more resistant to infection than older ones. The crowding that obtains in nursery plantings as they grow older probably also tends to make the host less resistant. Resistance has appeared to be associated with vigor. However, Stayman Winesap, which showed outstanding vigor on its own roots, was also outstandingly susceptible, showing a high percentage of infections as well as large lesions. This was true even during the first year of inoculations when the suppressive effect of crowding was less important than it later became.

These inoculations gave wide variation in percentage of infection and also in the average size of the lesions on the different clones. Some clones were more susceptible than others, but none was consistently highly resistant. This record points to the need for con-

tinued intensive testing of possible root stocks for use in areas where *Xylaria* abounds.

The yearly means (tables 5-7) show that the percentage of deep infection for all plots was comparatively low in 1936 and 1937, but much higher in 1938. The one inoculation experiment in 1939 also showed a high percentage of deep infection. The differences in the amount of infection in the different years are probably due to more than one concomitant factor. Earlier experiments have indicated that while the trees in a planting are still young and before competition and crowding are appreciable, a low percentage of infection often results from inoculation. Other contributing factors may have been temperature and moisture. Thermograph records in the field near the experimental plot showed that in 1936 there were only 15 days and in 1937 there were only 12 days from July 15 to September 1, the period after inoculation, that had a temperature as high as 80° F. (26.7° C.), in contrast to 25 days in 1938. Also the mean temperature for this period was only 76.8° F. (24.9° C.) in 1936 and 75.4° F. (24.1° C.) in 1937, while in 1938 it was 79.3° F. (26.3° C.). During the same period there were twice as many days having a daily mean temperature of 80° F. in 1938, the year of high infection, as in 1937, a year of low infection. The temperature studies reported on page 302 indicate that isolate 57, the strain of the fungus studied, is a high-temperature soil organism, the optimum in culture being about 25° C. An examination of the rainfall data for Beltsville, Md., for July and August, 1936, 1937, and 1938 gave no information that would explain the differences in infection in the different years.

These inoculation experiments agree in the main with the West Virginia experiments of Fromme and Schneiderhan (3), who reported that—

No evidence of any promising measure of resistance was exhibited by any one of 45 clons of *Malus* spp. exposed to natural infection, or by any one of 12 clonal stocks and 11 seedling stocks inoculated with *Xylaria mali* in pure culture.

#### GROWTH AND LONGEVITY OF *XYLARIA MALI* IN DEAD ROOTS

The question is often asked how long the black root rot fungus can remain alive in the soil and in the roots affected. Several different approaches to a study of the problem of longevity in *Xylaria mali* have been made.

In connection with other studies, at several times early in the summer, twenty 5-year-old apple trees were girdled just above the soil line by removing a zone of bark one-fourth inch wide and in midsummer the roots were inoculated with *Xylaria mali*. Where the roots died as a result of the girdling the lesions were smaller than where the girdled zone was partly closed over and the roots remained alive. Usually there was little enlargement of the *Xylaria* lesions after the death of the infected parts. Possibly harmful products from reactions in the killed host plant make conditions unfavorable for the growth of the fungus.

Other experiments to determine the longevity of *Xylaria* were made by cutting roots, known to contain the viable fungus, into 3-inch lengths. On November 17, 1933, over 100 sections from 38 roots from different infected trees were buried at depths of 3, 6, and 9 inches. After 4 months the viability of the fungus was determined



by placing the roots in moist chambers under conditions favorable for growth of the fungus and attempting to recover it by the usual isolation methods. Very few roots yielded cultures after 4 months, and after a year no viable mycelium was found. Undisturbed roots left in the soil for a year or more after the death of the host often gave negative results when attempts were made to isolate the pathogen. On the other hand, isolation of the pathogen from diseased roots was successful many times; in some cases the fungus was isolated from roots that had remained undisturbed in the soil for at least 4 years after the death of the host. Schneiderhan (5) reported a case in West Virginia where *Xylaria mali* remained alive in a root in the soil for 16 years.

These observations and experiments indicate that *Xylaria* often dies soon after the infected root dies. On the other hand, the pathogen may sometimes remain alive in some of the infected roots for a number of years after the death of the host.

A large number of isolation experiments as well as a study of infected roots in the soil and in moist chambers indicate that competing soil organisms may be important in killing *Xylaria mali* in apple roots. A species of *Trichoderma* was very frequently found growing on roots that had been killed by *Xylaria*, as evidenced by the characteristic stroma of *Xylaria*. Attempts to isolate the pathogen from the margins of the diseased areas of such roots often resulted in isolating *Trichoderma* but not *Xylaria*. Microscopic examination of the mycelium of *X. mali* in contact with the mycelium of a species of *Trichoderma* from apple roots in a mixed culture showed a collapse of the mycelium of *Xylaria*. The deleterious action of other soil organisms besides *Trichoderma* may be important in the killing of *X. mali*. Weindling (7) reported a lethal effect of *T. lignorum* on *Rhizoctonia solani*.

The origin of *Xylaria mali* is still obscure as is also its relation to other forms of the genus *Xylaria*. A number of different species of *Xylaria* occur throughout the world, but most of them are saprophytic. Lyon (4) reported an undetermined species of *Xylaria* as causing a root rot of hibiscus in Hawaii. *X. mali* is not known to occur on either wild or cultivated plants anywhere outside of the eastern and central part of the United States. This suggests that it is indigenous in this region, but a wild host has not been discovered. The wide variation in the pathogenicity of different isolates affords some evidence of transition stages from the saprophytic to the parasitic habit. These considerations suggest the hypothesis that this parasitic species developed from some of the related saprophytic forms present in the region where the disease occurs.

#### DISCUSSION

Black root rot is serious only in the southeastern and south-central parts of the United States. The regional distribution of the disease may be associated with soil temperature high enough to favor the growth of the pathogen, as indicated by temperature studies on one strain of the fungus.

Local distribution, on the other hand, appears to be at least partly dependent on host conditions. Orchard observations have indicated that the condition of the tree is important. Maturity of the tree and

also an unthrifty condition due to such conditions as overbearing, starvation, or cold injury appear to predispose to infection. Black root rot is essentially a disease of the bearing orchard. Young orchards are seldom affected, although young trees set where diseased ones have been removed usually become diseased. Young replants are subjected to two conditions that may explain their susceptibility: (1) There is an abundance of diseased roots from the removed tree to serve as inoculum; and (2) growing conditions for replants are not good because of competition with established trees and possibly also because of deficiencies of certain salts and an accumulation of toxic substances. Aside from the influence of the factors just mentioned, there are probably physiological changes that make the tree more susceptible after it reaches fruiting age. Overloading with fruit, such as often occurs when bearing is biennial, may result in a weakened tree. The physiological effect of overbearing has been observed by the writer on trees growing in the Pacific Northwest where winter injury of the sunscald type was much more severe on trees bearing an abnormally heavy load of fruit than on those that had been well thinned or that did not bear a crop that year. Similarly, a general weakened condition from overbearing might result in increased susceptibility to black root rot in sections where the disease prevails.

Although the inoculation experiments reported in this paper were carried out on trees of prebearing ages with no anticipation of the importance of age of the host, a review of the experiments shows that infection was more readily accomplished as the trees became older. On 4- to 6-year-old trees, inoculations usually resulted in a fair percentage of cases that made measurable lesions, but these lesions usually healed during the subsequent season. On younger trees, infection was more difficult to accomplish. However, in the region where the disease prevails, when once established on bearing trees, it continues to advance until the tree dies. Change in susceptibility to disease as the host becomes more mature has been shown in phytophthora trunk canker (1) and northwestern anthracnose (6) of apple.

Inoculation studies on young cherry, peach, plum, pear, and apple trees indicate that the apple is by far the most susceptible host. Small lesions have resulted from inoculation on cherry and pear trees, but they soon healed over. The results of inoculation experiments and of field observations indicate that the disease will probably not become serious on cherry, peach, plum, or pear trees growing in the vicinity of diseased apple trees.

Inoculation of a large number of scion and understock combinations with a virulent strain of the pathogen in pure culture indicates that there is sufficient variation in host susceptibility to justify the hope that resistance to the disease may be obtained by further search for resistant stocks.

#### SUMMARY

Black root rot of apple has a restricted distribution. It is not known to occur in any country except the United States. Here it is distributed from Pennsylvania to Georgia and westward to Arkansas. Distribution may be partly dependent on the soil temperature's being warm enough for the pathogen to thrive. Local distribution is apparently affected by certain conditions adverse to the host.

In inoculation experiments infection was not successful when the inoculum used was a mass of mycelium from an agar culture; it was successful when the inoculum was infected apple twigs. Field observations and inoculations indicate that wounding is not necessary for infection.

In agar cultures one isolate of *Xylaria mali* made fair growth at 15° C., but its optimum was about 25°. Roots of stored apple seedlings were infected at a temperature as low as 15.5°.

Inoculations with 27 different isolates of *Xylaria mali* indicate that there is wide variation in pathogenicity, as shown by the percentage of infection and the size of lesions produced on roots of yearling apple seedlings.

The relative susceptibility of the apple, pear, plum, cherry, and peach seedlings was determined by inoculation; only the apple showed undoubted susceptibility. Small lesions were produced on the pear and cherry, but these had healed over by the next year.

Tests were made for resistance to *Xylaria mali* in 3 or 4 successive years on 19 own-rooted standard apple varieties, on 30 seedling clones, and on seedlings of 9 named varieties of apples. Analysis of variance showed only slight significance for variety in own-rooted varieties; in the own-rooted seedling clones and variety seedlings there was no significance for variety. The year 1938 had higher temperatures than 1936 or 1937 for the period of 49 days subsequent to inoculations. Statistical analysis of the infection data showed a significant correlation between infection and season with high temperature.

Longevity of the fungus in infected apple roots was variable. In several experiments, the living fungus could not be found a few months after the death of the host. However, in some natural infections it survived the host by several years.

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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Awn Inheritance in Barley (Key No. Calif.-129) - - - - -	405
JAMES L. MYLER	
Insect Transmission of the Virus Causing Narcissus Mosaic (Key No. K-321) - - - - -	413
F. S. BLANTON AND F. A. HAASIS	
Asynaptic Gossypium Plants and Their Polyploids (Key No. Tex.-24) - -	421
J. O. BEASLEY AND META SUCHE BROWN	
The Influence of Feeding Low-Nitrogen Rations on the Reliability of Biological Values (Key No. N. Y. (Cornell)-52) - - - - -	429
J. I. MILLER AND F. R. MORRISON	



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## AWN INHERITANCE IN BARLEY<sup>1</sup>

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### INTRODUCTION

Awn inheritance in barley has long been of interest to barley geneticists. The awn or lemma projection on the barley spikelet shows many variations. Morphologically it is an extension of the vascular system of the lemma into a pointed appendage, which varies in different varieties from several inches in length to complete absence. These variations give rise to the various awn types, long-awned, short-awned, awnletted, and awnless. Most so-called awnless varieties, such as Arlington Awnless and Englawless, do show some awn development, particularly on the central spikelets. In some varieties the central spikelet is awned while the lateral spikelets are awnless, as in most two-row and intermediate (*Hordeum intermedium*) varieties. In six-row varieties the awns on the lateral spikelets are shorter than the central awns; they may be much reduced, or completely absent. Another variation in the lemma projection is the hood, in which the normal awn is replaced by a trifurcate structure. This hood, though commonly sessile, in certain varieties is elevated to varying degrees at the end of an awn.

### REVIEW OF LITERATURE

Several investigators have published material on awn inheritance. Their reports conflict somewhat, partly, at least, because certain difficulties of classification have tended to obscure the genetics.

Engledow<sup>3</sup> studied rather extensively the awn inheritance in a cross between a fully awned variety and Englawless. Fluctuations in awn length, caused by environment, complicated the problem of classification. Engledow's awnless variety, when planted late, developed up to one-half awns, whereas single, late-forming heads on plants otherwise completely awnless often produced one-fourth to one-half awns. From  $F_2$  data Engledow concluded that awned vs. awnless must be due to two factors; but on checking in  $F_3$  he concluded that only one factor was involved.

Kuckuck,<sup>4</sup> in reviewing the literature, cites work of Huber, who attributed awned vs. awnless to a single factor, the  $F_1$  being short awned, the  $F_2$  segregating 1 long-awned: 2 short-awned: 1 awnless.-Ubisch<sup>5</sup> reports a two-factor difference between long-awned and awnless, the factor  $J$  producing long awns, the recessive  $j$  producing short

<sup>1</sup> Received for publication November 26, 1941.

<sup>2</sup> Thanks are due Dr. F. N. Briggs for furnishing material, advice, and guidance throughout the experiments and during the preparation of this paper, and also to Dr. E. H. Stanford for valuable help in classifying and analyzing the results.

<sup>3</sup> ENGLEADOW, F. L. INHERITANCE IN BARLEY. III. THE AWN AND LATERAL FLORET: FLUCTUATION: A LINKAGE: MULTIPLE ALLELOMORPHS. Jour. Genet. 14: [49]-87, illus. 1924.

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<sup>5</sup> UBISCH, G. V. BEITRAG ZU EINER FAKTORENANALYSE VON GERSTE. Deut. Bot. Gesell. Ber. 41: 79-84. 1923.



awns. A second factor *S* causes awnless, though Ubisch makes no statement concerning the interaction of *J* and *S*.

Miyake and Imai<sup>6</sup> (Complete translation by Hiro Taketaya, senior Japanese student at the University of California) encountered much the same difficulty of classification as did Engledow. Their awnless variety developed short awns on late tillers. They reported a single-factor difference between long and short awns; a three-factor difference between a long-awned six-rowed variety and an awnless six-rowed variety. Although they made three classes—long-awned, short-awned, and awnless—the awn types were bulked to show the three-factor difference. In a cross of their awnless six-rowed variety with a long-awned two-rowed variety, they concluded that the two-rowed variety had four dominant factors  $A_1, A_2, A_3, A_4$ , for awn development, whereas the six-rowed awnless variety carried the recessives,  $a_1, a_2, a_3, a_4$ . Here, again, all awn types were bulked, though the investigators made a separation of the classes, long-awned, short-awned, and awnless. No  $F_3$  generation was grown to check  $F_2$  phenotypes.

Many workers<sup>7</sup> have reported hooded vs. awned as due to a single factor. Miyake and Imai<sup>8</sup> attributed it to a single factor; but hooded vs. awnless in a cross of their six-rowed awnless with a two-rowed hooded was reported as due to complementary factors. In this cross the  $F_2$  segregation was 9 hooded: 7 normal. The normal would presumably be awnless, but was not described by them. From the author's experience (presented later), the segregates in  $F_2$  from a cross of awnless with hooded would include, besides hooded and awnless, three awn lengths.

#### MATERIALS

Four long-awned, one awnless, and one hooded variety of barley (*Hordeum vulgare* L.) have been used in crosses thus far. The study of crosses involving other awn types is under way.

Atlas, the most important commercial variety of California, is a selection from Coast. It is a long-awned, six-rowed, lax type. Black Hull-less is a long-awned, six-rowed, lax, purple, naked type. An unnamed variety, C. I. No. 5628, is also a long-awned, six-rowed, lax purple, naked type. Another variety, Redrachis, C. I. No. 5649, is a two-rowed, lax type, with long smooth awns and a red rachis. Awnless, C. I. No. 5631, is an Atrada selection brought in by Dickson in 1932 from Russia. This variety is truly awnless, developing no awns, regardless of planting date or environmental factors at Davis, Calif. In addition, it is quite dense, has a short, stiff straw and matures fairly early. Nepal, a hooded, six-rowed, lax, naked variety, was the parent used in studying hooded vs. awnless. Although these varieties differ in other characters, the contrasting characters reported in this paper are shown in table 1.

<sup>6</sup> MIYAKE, KICHI, and IMAI, YOSHITAKA. GENETIC STUDIES IN BARLEY. I. Bot. Mag. [Tokyo] 36: 25-38. 1922. [In Japanese. English resumé, p. 27.]

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<sup>8</sup> See footnote 6.



TABLE 1.—*Parent varieties used in the study of awn inheritance*

Parent variety	C. I. <sup>1</sup> No.	Contrasting characters		
		Awn or hood type	Density	Rows
Awnless.....	5631	Awnless.....	Dense.....	6
Atlas.....	4118	Long-awned.....	Lax.....	6
Black Hull-less.....	666	do.....	do.....	6
Unnamed.....	5628	do.....	do.....	6
Redrachis.....	5649	do.....	do.....	2
Nepal.....	595	Hooded.....	do.....	6

<sup>1</sup> C. I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry U. S. Department of Agriculture.

## EXPERIMENTAL RESULTS

AWNED  $\times$  AWNLESS

The awn types as classified in  $F_2$  of the cross Atlas  $\times$  Awnless, showed 351 awned, 122 awnletted, and 32 awnless, which suggests a 12:3:1 ratio, although the fit is not satisfactory. The awnletted segregates had short awns on the central spikelets; and, though they varied somewhat, they tended to approach the awn type of Arlington Awnless. A two-factor basis for awn inheritance is further indicated by 473 awned: 32 awnless, where 473.4: 31.6 were expected on the basis of a 15: 1 ratio, and thus almost a perfect fit.  $F_3$  rows of about 30 plants each were grown, from which the  $F_2$  genotypes were determined.

The classification system was expanded to take care of the many breeding types encountered in  $F_3$  and to improve on the  $F_2$  classification, which, as pointed out above, was not entirely satisfactory. In view of the experience with awn shedding encountered in classifying  $F_2$  plants, the  $F_3$  generation was classified while the plants were still green. A close study in  $F_3$  revealed that plants formerly classed as long-awned could be divided into long- and short-awned. This had been overlooked in  $F_2$  because the actual length is influenced by head density (fig. 1). In very few cases were the lax plants as awnless as the awnless parent. The  $F_3$  classification showed that the nine genotypes expected on the basis of two factors were present in about the expected numbers (table 2). The four homozygous types—long-awned, short-awned, awnletted, and awnless—are shown in figure 1.

TABLE 2.—*Awn segregation in  $F_3$  rows of Atlas  $\times$  Awnless, grown at Davis, Calif., in 1939*

Type of segregation	Genotype	Observed	Expected	$\chi^2$
Long-awned.....	<i>LkLk Lk<sub>1</sub>Lk<sub>1</sub></i> .....	43	31.5	4.198
3 long-awned: 1 short-awned.....	<i>LkLk Lk<sub>1</sub>lk<sub>1</sub></i> .....	65	63.0	.063
3 long-awned: 1 awnletted.....	<i>Lklk Lk<sub>1</sub>Lk<sub>1</sub></i> .....	57	63.0	.571
All types.....	<i>Lklk Lk<sub>1</sub>lk<sub>1</sub></i> .....	130	126.0	.127
Short-awned.....	<i>LkLk lk<sub>1</sub>lk<sub>1</sub></i> .....	32	31.5	.008
3 short-awned: 1 awnless.....	<i>Lklk lk<sub>1</sub>lk<sub>1</sub></i> .....	53	63.0	1.587
Awnletted.....	<i>lklk Lk<sub>1</sub>Lk<sub>1</sub></i> .....	42	31.5	3.500
3 awnletted: 1 awnless.....	<i>lklk Lk<sub>1</sub>lk<sub>1</sub></i> .....	55	63.0	1.016
Awnless.....	<i>lklk lk<sub>1</sub>lk<sub>1</sub></i> .....	27	31.5	.643
Total.....	.....	504	504.0	11.713

The chi-square for the data in table 2 is 11.713, which gives a probability slightly below the 20-percent point. The two-factor explanation was further substantiated by a backcross of the  $F_1$  to Atlas, which yielded the table 3 data in  $F_1$  as determined from the segregation in  $F_2$  rows.



FIGURE 1.—The four true breeding awn types, long-awned (A), short-awned (B), awnletted (C), and awnless (D), as they appear on the lax and dense spikes, respectively.

TABLE 3.—Awn segregation in  $F_2$  of the backcross of Atlas  $\times$  Awnless to Atlas, grown at Davis, Calif., in 1939

Type of segregation	Genotype	Observed	Expected	$\chi^2$
Long-awned.....	<i>LkLk Lk<sub>1</sub>Lk<sub>1</sub></i> .....	6	7.75	0.395
3 long-awned: 1 short-awned.....	<i>LkLk Lk<sub>1</sub>lk<sub>1</sub></i> .....	8	7.75	.008
3 long-awned: 1 awnletted.....	<i>Lklk Lk<sub>1</sub>Lk<sub>1</sub></i> .....	9	7.75	.202
All types.....	<i>Lklk Lk<sub>1</sub>lk<sub>1</sub></i> .....	8	7.75	.008
Total.....		31.0	31.00	.613

The four classes (table 3) expected in the backcross should occur in equal numbers. The chi-square value of 0.613 shows an excellent fit to the expected, with a probability above 0.8. Atlas differs, therefore, from Awnless in two major dominant factors (*LkLk Lk<sub>1</sub>Lk<sub>1</sub>*) for awn development, both of which are necessary for the development of long awns. One dominant, *Lklk<sub>1</sub>*, alone produces short awns, whereas the other, *lkLk<sub>1</sub>*, gives the awnletted condition.

Additional data concerning awn inheritance are available from three other crosses, grown in part for other purposes. These will be considered briefly.

The first, Awnless  $\times$  Black Hull-less, as classified in  $F_2$  showed 297 long-awned, 256 short-awned, 237 awnletted, and 39 awnless, where 466.3, 155.4, 155.4, and 51.8, respectively, were the numbers expected on the basis of two factors. Here, again, the poor fit was due to difficulties in classification. The awn in Balck Hull-less is somewhat longer than in Atlas although both are considered long-awned. The heterozygous types in this cross tended to be somewhat more intermediate than in the Atlas cross. Plants heterozygous for both factors were classified about equally in the long- and short-awn classes, a few being classified as awnletted, as later shown by the  $F_3$ . Many plants heterozygous for long-awned and awnletted ( $Lklk$ ,  $Lk_1Lk_1$ ) were classified as short-awned; and plants of the genotype ( $Lklk$ ,  $lk_1lk_1$ ) which in  $F_3$  segregate 3 short-awned: 1 awnless were often classified as awnletted.

Four hundred rows of the  $F_3$  generation of this cross were grown and classified. The  $F_2$  generation of those 400 plants showed 154 long-awned, 122 short-awned, 108 awnletted, and 16 awnless, where 225, 75, 75, and 25 were expected. This is a very poor fit to the expected 9:3:3:1 ratio. The results from the  $F_3$  classification are shown in table 4, and conform to expectations.

TABLE 4.—Awn segregation in  $F_3$  rows of Black Hull-less  $\times$  Awnless, grown at Davis, Calif., in 1941

Type of segregation	Genotype	Observed	Expected	$\chi^2$
Long-awned	$LkLk Lk_1Lk_1$	34	25	3.240
3 long-awned: 1 short-awned	$LkLk Lk_1lk_1$	49	50	.020
1 long-awned: 2 short-awned: 1 awnletted	$Lklk Lk_1Lk_1$	49	50	.020
All types	$Lklk Lk_1lk_1$	103	100	.090
Short-awned	$LkLk lk_1lk_1$	29	25	.640
1 short-awned: 2 awnletted: 1 awnless	$Lklk lk_1lk_1$	39	50	2.420
Awnletted	$lklk Lk_1Lk_1$	97	100	.090
3 awnletted: 1 awnless	$lklk Lk_1lk_1$			
Awnless	$lklk lk_1lk_1$			
Total		400	400	6.520

The chi-square value of 6.52 shows a probability of between 0.3 and 0.5. Thus the data in table 4 show clearly that in this, as in the Atlas cross, awn inheritance depends upon two factors. Plants heterozygous for long-awned and awnletted, though usually classified as short-awned, may be distinguished from homozygous short-awned plants because the awns on the central spikelets are coarser and tend to be much longer than the awns on the lateral spikelets.

When the  $F_3$  data are used to correct the  $F_2$  classification, there are found to be 235 long-awned: 68 short-awned: 80 awnletted: 17 awnless, where 225: 75: 75: 25 are expected, giving a  $P$  value greater than 0.2.

An  $F_2$  population of 496 plants of C. I. No. 5628  $\times$  Awnless was classified in 1941 for awn inheritance. The author, having become more familiar with the heterozygous types from classifying the  $F_3$  rows of Black Hull-less  $\times$  Awnless, was able to effect a satisfactory classification in  $F_2$  of this cross, making it unnecessary to grow the  $F_3$ . The  $F_2$  data are shown in table 5.

TABLE 5.—Awn segregation in  $F_2$  of the cross C. I. No. 5628  $\times$  Awnless, grown at Davis, Calif., in 1941

Phenotype	Observed	Expected	$\chi^2$
Long-awned.....	273	279	0.129
Short-awned.....	99	93	.387
Awnletted.....	99	93	.387
Awnless.....	25	31	1.161
Total.....	496	496	2.064

With a probability between 0.5 and 0.7, the data in table 5 agree with those for the two previously discussed crosses; and apparently C. I. No. 5628 carries the same awn factors as Atlas and Black Hull-less.

An  $F_2$  population of 352 plants in the cross of two-rowed, long-awned Redrachis  $\times$  Awnless was grown in 1941. Since the heterozygous types must be identified from the difference in length of central and lateral spikelets, it was not possible in this cross to separate these types. The  $F_2$  plants were classified, accordingly, as awned or awnless; this system showed 323 awned: 29 awnless, giving a chi-square value of 2.375 and a probability between 0.1 and 0.2 for a 15:1 ratio. These results do not agree with the results of Miyake and Imai,<sup>9</sup> who showed a four-factor difference between a six-rowed awnless and a two-rowed awned variety. The varieties used by them, however, are not the same varieties used in this study.

HOODED  $\times$  AWNLESS

In the cross, hooded Nepal  $\times$  Awnless, the  $F_2$  segregation shows all the awn types that occur in the cross awned  $\times$  awnless, in addition to hooded segregates.

The same difficulties were encountered in classifying this cross as in the previous crosses, density again affecting awn length. The awnletted and awnless types were combined because of the difficulty of classification, particularly in  $F_2$  populations. An  $F_2$  population of 882 plants yielded the results shown in table 6.

TABLE 6.—Segregation for hooded, awned, and awnless in the cross Nepal  $\times$  Awnless, grown at Davis, Calif., in 1940

Phenotypes	Dominant genes	Observed	Expected	$\chi^2$
Hooded.....	$LkLk_1 K$ .....	372	372.1	0.000
Long-awned.....	$LkLk_1 k$ .....	109	124.0	1.815
Short-awned.....	$lklk_1 (K, \text{ or } k)$ .....	162	165.4	.070
Awnletted.....	$lkLk_1 (K, \text{ or } k)$ .....	239	220.5	1.552
Awnless.....	$lklk_1 (K, \text{ or } k)$ .....			
Total.....		882	882.0	3.437

The chi-square for the data in table 6 is 3.437, which gives a probability between 0.3 and 0.5. This explanation assumes that both factors for awn development must be present before a hood can develop, and agrees with the previous explanation for awn inheritance. In this cross, Nepal carries both factors for awn development in addition to

<sup>9</sup> See footnote 6.



the hood factor, which masks awn development. This explanation differs from the results reported by Miyake and Imai<sup>10</sup> from a cross of their six-rowd awnless  $\times$  two-row hooded. They report complementary factors for hooded vs. normal, with hooded dominant.

The  $F_3$  generation of this cross was planted in the fall of 1940, and 416 rows were classified in the spring of 1941. Table 7 shows the results of the  $F_3$  classification.

TABLE 7.—Segregation in  $F_3$  rows for hooded, awned, and awnless in the cross Nepal  $\times$  Awnless, grown at Davis, Calif., in 1941

Type of segregation	Genotype	Observed	Expected	$\chi^2$
Hooded.....	$LkLk Lk_1Lk_1 KK$	8	6.5	0.346
3 hooded: 1 long-awned.....	$LkLk Lk_1Lk_1 Kk$	14	13.0	.077
3 hooded: 1 short-awned.....	$LkLk Lk_1lk_1 KK$	16	13.0	.692
3 hooded: 1 awnletted.....	$Lklk Lk_1Lk_1 KK$	20	13.0	3.769
9 hooded: 3 long-awned: 4 short-awned.....	$LkLk Lk_1lk_1 Kk$	12	26.0	7.538
9 hooded: 3 long-awned: 4 awnletted.....	$Lklk Lk_1Lk_1 Kk$	16	26.0	3.846
9 hooded: 3 short-awned: 3 awnletted: 1 awnless.....	$Lklk Lk_1lk_1 KK$	38	26.0	5.538
27 hooded: 9 long-awned: 12 short-awned: 12 awnletted: 1 awnless.....	$Lklk Lk_1lk_1 Kk$	50	52.0	.077
Long-awned.....	$LkLk Lk_1Lk_1 kk$	15	6.5	11.115
3 long-awned: 1 short-awned.....	$LkLk Lk_1lk_1 kk$	22	13.0	6.231
3 long-awned: 1 awnletted.....	$Lklk Lk_1Lk_1 kk$	17	13.0	1.231
9 long-awned: 3 short-awned: 3 awnletted: 1 awnless.....	$Lklk Lk_1lk_1 kk$	25	26.0	.038
Short-awned.....	$LkLk lk_1lk_1 Kk$	30	26.0	.615
	$LkLk lk_1lk_1 Kk$			
	$LkLk lk_1lk_1 kk$			
3 short-awned: 1 awnless.....	$Lklk lk_1lk_1 Kk$	39	52.0	3.250
	$Lklk lk_1lk_1 Kk$			
	$Lklk lk_1lk_1 kk$			
Awnletted.....	$lklk Lk_1Lk_1 Kk$	29	26.0	.346
	$lklk Lk_1Lk_1 Kk$			
	$lklk Lk_1Lk_1 kk$			
3 awnletted: 1 awnless.....	$lklk Lk_1lk_1 Kk$	43	52.0	1.558
	$lklk Lk_1lk_1 Kk$			
	$lklk Lk_1lk_1 kk$			
Awnless.....	$lklk lk_1lk_1 Kk$	22	26.0	.615
	$lklk lk_1lk_1 Kk$			
	$lklk lk_1lk_1 kk$			
Total.....		416	416.0	46.882

The chi square for the data in table 7 shows a poor fit to the expected, with a  $P$  value below 0.01. The homozygous long-awned rows, about which there could be little chance for error in classification, contribute almost one-fourth of the total chi square. The number of plants per row was too small for accurate determination of such ratios as 9 hooded: 3 long: 4 short; but the number of rows obtained is reasonably close to that expected. When the  $F_2$  was classified on the basis of  $F_3$ , there were 174 hooded, 79 long-awned, 69 short-awned, 72 awnletted, and 22 awnless, where 175.5, 58.5, 78.0, 78.0, and 26.0 were expected if Nepal carries both awn factors in addition to the hooded factor. This gives a chi-square value of 9.312 and a  $P$  value between 0.05 and 0.1.

As the  $F_2$  and  $F_3$  data clearly show, Nepal differs from Awnless in the three factors  $LkLk$ ,  $Lk_1Lk_1$ , and  $KK$ , and both dominant awn factors must be present for the expression of hoods. In several cases, however, a "rudimentary hood" developed on the short-awned and awnletted plants. In the short-awned plants it took the form of a flat, somewhat wider area in the awn, at which point the awn tended to bend or twist. In the awnletted plants it tended to cause a minute fork on the tip of a few of the awnletted central spikelets. These

<sup>10</sup> See footnote 6.

were not classed as hoods because they did not develop uniformly and were much less numerous than would be expected on the basis of a reasonable genetic interpretation.

#### SUMMARY

Awn inheritance in the cross Atlas  $\times$  Awnless was shown to be due to two factors, *Lklk* and *Lk<sub>1</sub>l<sub>1</sub>*. Long-awned plants carry both dominants. Short-awned plants have the first factor, *Lk*, dominant and the second, *Lk<sub>1</sub>*, recessive. Awnletted plants have the first factor, *Lk*, recessive and the second, *Lk<sub>1</sub>*, dominant. Plants carrying both recessives are awnless. This two-factor explanation is also true in the crosses Black Hull-less  $\times$  Awnless and C. I. No. 5628  $\times$  Awnless. In the two-rowed, long-awned, Redraxis  $\times$  Awnless cross, a two-factor difference was shown with a 15:1 ratio.

In the Nepal  $\times$  Awnless cross it was shown that these varieties differ by three factors for awn development. Nepal carries both awn factors, *LkLk* and *Lk<sub>1</sub>Lk<sub>1</sub>*, besides the factor *KK* for hood development. Awnless carries the three recessives. For the development of hooded plants, both dominant awn factors must be present in addition to the hood factor. Thus in  $F_2$  the expected segregation is 27 hooded: 9 long-awned: 12 short-awned: 12 awnletted: 4 awnless. The hood factor, *KK*, in a few cases tends to flatten and to cause a bend or twist near the end of the short awns. It also tends to cause a minute fork at the tip of the awnlet of a few central spikelets in the awnletted segregates.

## INSECT TRANSMISSION OF THE VIRUS CAUSING NARCISSUS MOSAIC<sup>1</sup>

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### INTRODUCTION

The mosaic disease of narcissus is known to be caused by a transmissible virus (9, 12, 13),<sup>2</sup> but the method by which the virus is disseminated has remained obscure. Several theories have been advanced, including the possibility of insects being either directly or indirectly concerned. Darlington (7) suggested that the disease may be caused through root injury resulting from larval feedings of the swift moth. Hodson (10) considered *Thrips tabaci* Lind. to be a likely vector of the virus, but according to Smith (14, p. 391), Hodson later claimed that evidence is against such an hypothesis and considered *Tarsonemus laticeps* Halbert (*T. approximatus* var. *narcissi* Banks) as worthy of consideration as a vector. In a later report Hodson (11) mentioned this mite as well as the mosaic disease of narcissus, but said nothing of the mite's being a probable vector of the virus; so presumably the theory was abandoned.

Following a field survey on Long Island, Blanton (2) reported that 20 species of leafhoppers and related Homoptera had been observed feeding on narcissus. In another paper (3) he listed 5 species of thrips from narcissus. Subsequently the clover mite (*Eryobia praetiosa* Koch) and the springtail *Bourletiella hortensis* (Fitch) were found on this plant. In addition, 7 species of aphids feeding on narcissus foliage have been collected—*Macrosiphum solanifolii* (Ashm.), *M. taraxaci* (Kalt.), *M. pisi* (Kalt.), *Aphis rumicis* L., *Myzus persicae* (Sulz.), *Anuraphis roseus* (Baker), and *Brevicoryne brassicae* (L.).

Several workers (1, 6, 11, 12) have questioned the association of aphids, the usual vectors of mosaic pathogens, with the natural dissemination of the virus causing narcissus mosaic. The present writers (4, 5), however, reported in recent notes that the aphid species *Macrosiphum solanifolii*, *M. pisi*, *M. rosae* (L.), *Aphis rumicis*, *Myzus convolvuli* (Kalt.), *M. cerasi* (F.), and *Anuraphis roseus* are capable of transmitting the virus. It is the purpose of this paper to present a detailed account of this work as well as of experiments with other possible vectors, including several species of thrips and leafhoppers, the springtail previously mentioned, the clover mite, and the bulb scale mite (*Tarsonemus laticeps*).

<sup>1</sup> Received for publication December 30, 1941.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 419.

## MATERIALS AND METHODS

Several varieties of virus-free narcissus plants were used in the transmission tests. All these plants were grown on the laboratory grounds for 2 or more years before being used in the experiments. During this period the plants were inspected early in the season, and all plants that appeared to be infected were removed. Mosaic-infected Sir Watkin plants served as the source of virus inoculum for all the tests. After being used for this purpose, they were isolated and grown for another year to check the first diagnosis.

With the exception of *Frankliniella fusca* (Hinds), a stock colony of which was obtained from J. G. Watts of the South Carolina Agricultural Experiment Station, all insect and mite species were collected from various host plants in the vicinity of narcissus plantings in Babylon, N. Y. These collections also served as the source for some of the aphid species established in pure-line colonies, which were reared after single aphids had been manually transferred to suitable host plants confined in cheesecloth cages.

Tests were conducted both in the greenhouse and in the field. Prior to 1935 healthy test plants were grown singly in 8-inch porous-clay pots in the greenhouse, to avoid the possibility of virus transfer by root contact. Haasis (9), however, demonstrated from experiments conducted in 1934 that virus transfer in this manner was unlikely; so in 1935 and subsequent years healthy and mosaic plants were grown together in either pots or flats, or in field plots. Each flat and each field plot contained from 20 to 24 plants in the ratio of approximately 1 diseased plant to 4 healthy plants, and the flats and plots were sometimes replicated several times.

Most of the plants grown in pots were enclosed in celluloid cylinders according to technique described elsewhere (9). For tests with the bulb scale mite, pots containing a diseased and a healthy plant were placed in a greenhouse where no other narcissus plants were being grown and were isolated from each other by means of a water barrier. The flats and field plots were enclosed in cages covered with fine-mesh cheesecloth.

In the tests with plants grown singly in pots, the insects were fed upon the mosaic-diseased plants for a definite period and then transferred to the cages containing the healthy plants. As a check, insects of the same species were fed for the same time on healthy narcissus and then transferred to other healthy narcissus. In the tests with diseased and healthy plants confined together, the insects were transferred to these plants directly from the host plant. All tests with aphids (with one exception as shown in table 1), thrips, and one mite were handled in this manner. This mite and the thrips were allowed to remain until all the plants died, being free to feed on both healthy and diseased plants. These species increased enormously, several generations being involved. The life cycle was complete, all stages being present when the plants died. All the aphids, however, died a few days after being confined with narcissus. A number of field plots and some flats of bulbs were caged, and thus kept free of insects, to serve as a second set of checks in some of the transmission tests involving aphids.



## RESULTS

The results of the transmission tests with miscellaneous insects and mites are given in tables 1 and 2, and those with aphids in table 3. The data for the check flats and plots from which insects were excluded are given in table 4.

TABLE 1.—Transmission of the virus causing narcissus mosaic by insects and mites when transferred from mosaic plants, and from healthy plants, to individually potted healthy *Sir Watkin* narcissus plants, 1932-34

Species	Host plant	Time confined before transfer to healthy plants	Tests with insects transferred from mosaic to healthy plants				Tests with insects transferred from one healthy plant to another (checks)			
			Time confined on healthy plants	Insects per plant	Healthy plants exposed	Plants developing infection	Time confined after transfer	Insects per plant	Plants exposed	Plants developing infection
		Days	Days	Number	Number	Number	Days	Number	Number	Number
<i>Aceratagallia sanguinolenta</i> (Prov.)	Clover ( <i>Trifolium</i> sp.)	34	15	10	5	0	15-18	10	5	0
<i>Macrostelus divisus</i> (Uhl.)	Ragweed ( <i>Ambrosia</i> sp.)	3	5	10	5	0	8-10	10	5	0
<i>Dikraneura</i> sp.	Quackgrass ( <i>Agropyron repens</i> (L.) Beauv.)	3	3-6	10	5	0	4-9	10	5	0
<i>Draeculacephala molipes</i> (Say)	do.	2	1-2	10	5	0	3	10	5	0
<i>Stobaera tricarinata</i> (Say)	Ragweed ( <i>Ambrosia</i> sp.)	2	4-6	3-6	5	0	3-6	3-6	5	0
<i>Bourletiella hortensis</i> (Fitch)	Clover ( <i>Trifolium</i> sp.)	2-3	5	15-20	5	0	5	15-20	5	0
<i>Bryobia praetiosa</i> Koch.	Quackgrass ( <i>Agropyron repens</i> )	2-3	5	10-20	5	0	2-10	10-20	5	0
<i>Aphis rumicis</i> L.	Dock ( <i>Rumex</i> sp.)	2	3	Many	5	3	3-6	Many	5	0

TABLE 2.—Transmission of the virus causing narcissus mosaic by thrips and mites when confined throughout their life cycle with diseased and healthy *Sir Watkin* narcissus plants potted together, 1935

Species	Host plant	Insects confined with a mosaic and a healthy plant			Insects confined with healthy plants only (checks)		
		Insects per plant	Healthy plants exposed	Plants developing infection	Insects per plant	Healthy plants exposed	Plants developing infection
<i>Frankliniella fusca</i> (Hinds)	Cotton ( <i>Gossypium</i> sp.)	Number 10-15	Number 29	Number 0	Number 50	Number 5	Number 0
<i>Thrips tabaci</i> Lind.	Onion ( <i>Allium</i> sp.)	5-25	51	0	3-20	19	0
<i>Tarsonemus laticeps</i> Halbert.	Narcissus <i>Narcissus pseudonarcissus</i> L.)	Many	3	0			

<sup>1</sup> Insects remaining through several generations, the life cycle being complete.

TABLE 3.—Transmission of the virus causing narcissus mosaic by 7 species of aphids when confined with plants in flats or field plots, 1935-39

Species	Host plant on which aphids developed	Variety of narcissus	Location of plants	Date of transfer of aphids to healthy plants	Tests with aphids transferred from one healthy plant to another (checks)		Tests with aphids transferred from one healthy plant to another (checks)	
					Plants exposed	Plants developing infection	Plants exposed	Plants developing infection
<i>Macrosiphum solanifolii</i> (Ashm.) <sup>1</sup>	Potato ( <i>Solanum tuberosum</i> L.)	{ Sir Watkin King Alfred Sir Watkin Minister Talma Subtotal	Greenhouse  do. Field do.	{ Feb. 2	Number 70	Number 14	Number 0	
				{ Feb. 10	72	32	23	
				{ Feb. 17	70	27	24	
				{ Mar. 4	71	29	46	
				{ Mar. 9	24	24	20	
<i>Macrosiphum rosae</i> (L.)	Rose ( <i>Rosa</i> sp.)	{ Sir Watkin King Alfred Minister Talma Subtotal	Field do. do.	{ May 1-10	38	8	1	
				{ do.			53	
					403	152	17	
				{ May	20	12	280	
				{ May 1-10	58	36		
<i>Macrosiphum pisi</i> (Kalt.)	Vetch ( <i>Vicia</i> sp.)	{ Sir Watkin King Alfred Minister Talma Subtotal	Field do. do.	{ May	54	41	2	
				{ May				
					136	89	54	
				{ May 1-10	31	24		
<i>Aphis rumicis</i> L.	Nasturtium ( <i>Tropaeolum</i> sp.) <sup>1</sup> or dock ( <i>Rumex</i> sp.)	{ Sir Watkin Minister Talma King Alfred Spring Glory Victoria Subtotal	{ Greenhouse do. Field do. do. Greenhouse do.	{ Feb. 2	21	5		
				{ Feb. 10	22	8	23	
				{ Apr. 30	277	240	119	
				{ May 1-10	58	40	40	
				{ do.	39	12	17	
<i>Myzus convolvuli</i> (Kalt.)	Tulip ( <i>Tulipa</i> sp.)	{ Sir Watkin King Alfred Subtotal	Greenhouse do.	{ do.	80	46	2	
				{ May	24	6	15	
				{ do.	84	41	12	
					605	308	0	
<i>Myzus cerasi</i> (F.)	Cherry ( <i>Prunus</i> sp.)	{ Sir Watkin King Alfred Subtotal	Greenhouse do. do.	{ Mar. 4	48	25	14	
				{ Mar. 30	233	157	20	
				{ Mar. 9	43	28	66	
					324	210	24	
<i>Anuraphis roseus</i> (Baker)	Apple ( <i>Malus</i> sp.)	{ Sir Watkin do Total	Field do.	{ June 5	36	15	9	
				{ June 1	18	13		
					1,553	901	655	

<sup>1</sup> Pure-line colonies.

TABLE 4.—*Development of mosaic disease in narcissus plants in field plots or flats that were caged to exclude insects, 1935-39*

Variety of narcissus	Location of plants	Plants caged	Plants developing infection
		Number	Number
Sir Watkin.....	Field.....	106	4
King Alfred.....	do.....	74	5
Victoria.....	Greenhouse.....	51	6
	do.....	12	0
Total.....		243	15

In the early tests with miscellaneous insects negative results were obtained with all species except *Aphis rumicis*. Since this species transmitted the virus to three of the five healthy narcissus plants exposed to inoculation, and since the check plants remained healthy, all transmission trials are considered as comparable. In the later tests with aphids all seven species transmitted the mosaic virus, but there was considerable variation in the proportion of plants infected.

A few of the check plants from which insects were excluded also became infected, but the number was very small compared with that of plants containing aphids. The cause of these accidental infections is uncertain, but two possibilities are suggested: (1) Some of the supposedly healthy plants on which the colonies were maintained may have served as symptomless carriers of the virus; (2) migratory aphids, having acquired the virus by feeding on mosaic narcissus growing near the cages, may have made a feeding contact with the healthy foliage through the cheesecloth barriers. The writers believe the second possibility to be the more tenable, for they did observe a few migratory aphids feeding through the cheesecloth. Furthermore, 18 of the 22 accidental transmissions occurring in the caged field plots were found to be in plants next to the periphery of the cage and the remaining 4 were in the adjacent row, approximately 10 inches from the edge of the cage. If these accidental transmissions resulted from inoculations within the cages, a more random distribution of the infected plants should be expected. The locations of infected plants in the greenhouse were not charted, but the majority of the accidental transmissions occurred in plants adjacent to the edge of the cage.

A total of 904 plants were infected as a result of inoculation by aphids, but none showed morphologic symptoms of the mosaic disease during the growing season in which the inoculations were made. Similar results are reported where inoculations were performed mechanically (9, 12, 13).

The experimentally infected plants used in these tests expressed both the chlorotic-striping and leaf-enation symptoms that are characteristic of the disease as exhibited by naturally infected plants of the same variety growing in the field.

#### DISCUSSION

Narcissus plantings in Long Island are first invaded by aphids in the latter part of April, when these insects commence their migrations

from primary to secondary hosts, and from this early date until the narcissus plants mature light populations persist. It appears that narcissus serves only as a transient host for the aphids, for under controlled conditions, when aphids are restricted to a narcissus diet while the plants are still growing, the period of survival ranges from 3 to 15 days. The species *Macrosiphum solanifolii*, however, is exceptional in that it forms colonies and multiplies rapidly on narcissus foliage, beginning the latter part of June.

Of the seven aphid species that have been taken on narcissus in the field, the three most frequently encountered have been *Macrosiphum solanifolii*, *M. pisi*, and *Aphis rumicis*; *Anuraphis rosae* was abundant only one year during these investigations. All four species transmitted the virus under experimental conditions (table 3). Three additional species, *M. rosae*, *Myzus convolvuli*, and *M. cerasi*, although never collected from narcissus, are also efficient vectors. These facts suggest that the virus causing narcissus mosaic may be disseminated by many species of aphids, as has similarly been demonstrated for the yellow dwarf virus of onions (8).

In most of the transmission tests with aphids from 100 to 500 insects per cage were employed. With the exception of *Macrosiphum solanifolii* late in the growing season, aphid populations on narcissus under field conditions rarely if ever reach such high numbers. Preliminary data are at hand, however, which indicate that a single aphid is able to transmit the virus. In such a case even sparse populations could account for the high percentage of virus dissemination that takes place in the field.

#### SUMMARY

Investigations were conducted to determine whether any of the insects collected from field-grown narcissus plants are capable of transmitting the virus causing narcissus mosaic.

Fifteen species of insects and two species of mites were allowed to feed on mosaic narcissus plants and were then transferred to healthy narcissus, which were maintained in cages in both the greenhouse and the field. The results were negative for the miscellaneous insects and mites, which included five species of leafhoppers, *Aceratagallia sanguinolenta* (Prov.), *Macrosteles divisus* (Uhl.), *Dikraneura* sp., *Draeculacephala mollipes* (Say), *Stobaera tricarinata* (Say); two species of thrips, *Frankliniella fusca* (Hinds) and *Thrips tabaci* Lind.; one springtail, *Bourletiella hortensis* (Fitch); and two species of mites, *Bryobia praetiosa* Koch and *Tarsonemus laticeps* Halbert. All seven species of aphids—*Macrosiphum solanifolii* (Ashm.), *M. rosae* (L.), *M. pisi* (Kalt.), *Aphis rumicis* L., *Myzus convolvuli* (Kalt.), *M. cerasi* (F.), and *Anuraphis roseus* (Baker)—gave positive results.

Four of the aphid species—*Macrosiphum solanifolii*, *M. pisi*, *Anuraphis roseus*, and *Aphis rumicis*—have been collected on narcissus plants growing in the field, but only *M. solanifolii* has been found capable of multiplying on these plants.

The aphids transmitted the virus to 904 out of a total of 1,558 plants of the following narcissus varieties: Sir Watkin, King Alfred, Minister Talma, Spring Glory, and Victoria.



Symptoms of the disease appeared during the season following inoculation and were typical of the disease as exhibited by naturally infected plants of the same variety growing in the field.

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# ASYNAPTIC GOSSYPIUM PLANTS AND THEIR POLYPLOIDS<sup>1</sup>

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## INTRODUCTION

In many hybrids between distantly related species chromosomes fail to pair during meiosis or pairing is incomplete (4, 5)<sup>2</sup>. There are several known examples in which chromosomes fail to pair in non-hybrid individuals or in segregates from hybrids that usually give normal chromosome pairing (1, 2, 4, 5, 9, 10). Doubling the chromosome number in hybrids in which most of the chromosomes fail to pair is usually followed by nearly normal chromosome pairing. From the work of Dobzhansky (5) doubling the chromosome number in nonhybrid individuals with reduced chromosome pairing, or in hybrids in which chromosomes have a reduced pairing as a result of gene combinations, would be expected to give no increase in the number of chromosomes paired.

Sterile plants were reported by Kearney (8) to segregate in the  $F_2$  and  $F_3$  of *Gossypium hirsutum*  $\times$  *G. barbadense*, both of which are American cultivated tetraploid cottons with 26 pairs of chromosomes. Sterile plants from this cross have also been noted by Harland (6) and by others who have worked with the cross. Sterile plants were reported by Hutchinson and Gadkari (7) to segregate in a ratio of 3 fertile to 1 sterile in an Asiatic cotton with 13 pairs of chromosomes.

## MEIOTIC CHROMOSOME BEHAVIOR IN ASYNAPTIC PLANTS AND THEIR POLYPLOIDS

In  $F_2$  populations of *Gossypium hirsutum*  $\times$  *G. barbadense*, plants were found that flowered but produced no seeds.<sup>3</sup> Anthers of some flowers dehisced, but examination with a hand lens indicated that the pollen was aborted. Apparently no functional embryo sacs or pollen were produced. Although some fruits reached nearly mature size, they had no seeds. Acetocarmine smears of pollen mother cells of  $F_2$  plants show 26 pairs of chromosomes with no evidence of structural differences between the chromosomes (3), and fertile  $F_2$  plants have the same meiotic chromosome behavior. In meiosis of the sterile plants less than half of the 52 chromosomes paired (fig. 1). The range in number of pairs was 2 to 15, and means of different sterile  $F_2$  plants ranged from 6.3 to 11.6 (table 1). The chromosomes that paired usually had only one chiasma, and the univalents were scattered around the metaphase plate. At anaphase some bivalents separated sharply like normal bivalents with one chiasma, but some

<sup>1</sup> Received for publication, January 3, 1942.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 427.

<sup>3</sup> The first of the sterile plants used in this work were found in an  $F_2$  progeny grown by Dr. Thomas Kerr, who also aided in examining the plants for sterility. Data were collected from other  $F_2$  populations grown by T. R. Richmond; Dr. C. P. Swanson aided in the cytological work.

became attenuated. No fragments were found. At anaphase multipolar spindles frequently were present. In the second division the chromosomes formed regular spindles and apparently separated as

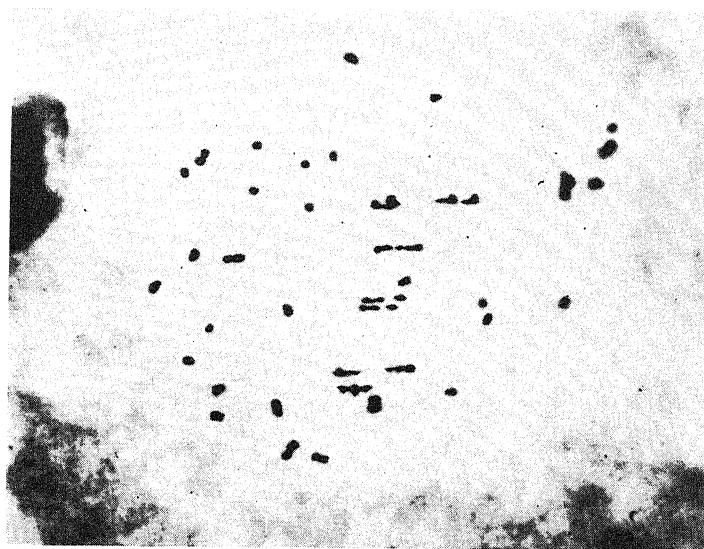


FIGURE 1.—First meiotic metaphase of sterile plant from  $F_2$  progeny of *Gossypium hirsutum*  $\times$  *G. barbadense* showing less than one-third of the chromosomes paired.

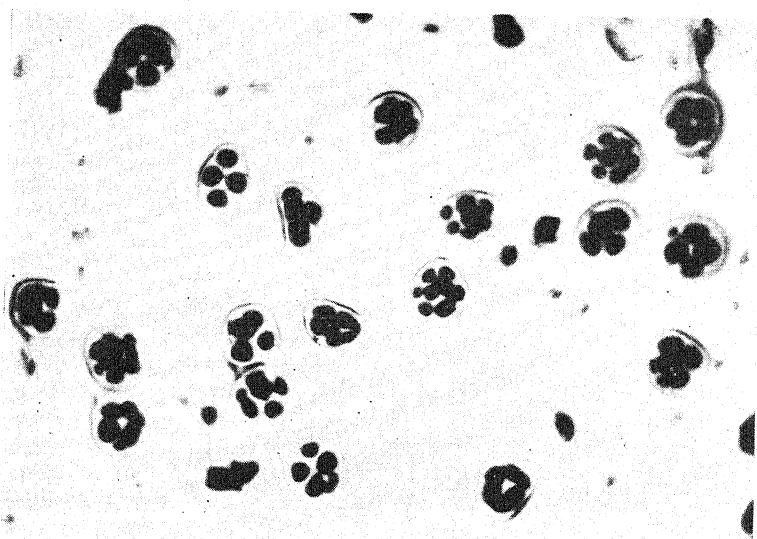


FIGURE 2.—Microsporocytes of sterile plant from  $F_2$  progeny of *Gossypium hirsutum*  $\times$  *G. barbadense* showing more than four microspores in "tetrads."

they do in normal plants. This meiotic chromosome behavior resulted in "tetrads" with 1 to 13 microspores (table 2) with extreme variability in size (fig. 2).



TABLE 1.—Number of pairs of chromosomes in  $F_2$  asynaptic plants from *Gossypium hirsutum*  $\times$  *G. barbadense*

Plant No. <sup>1</sup>	Chromosome number	Pairs of chromosomes																																	Number of nuclei	Mean
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
1.....	4N					1	4	8	9	11	7	5	4																						40	8.8
1 treated.	8N																																		44	23.7
2.....	4N					1	7	10	7	6	6	4		1																					43	9.0
2 treated.	8N																																		24	24.7
3.....	4N																																		40	11.6
4.....	4N					3			1	5	3	7	10	7	5	1																			15	7.9
5.....	4N																																		17	8.2
6.....	4N					2	3	12	4	3	3	1	2	1																					28	7.0
7.....	4N					3	3	12	4	3	2																								40	6.4
8 <sup>1</sup> .....	4N					2	7	3	4	2	6																								25	6.7
9.....	4N					3			2	6	7	6	3																						24	8.8
10.....	4N					5	1	10	5	6	2	1	3	1																					24	6.8
11.....	4N					9	10	10	4	6	2	1	3	2																					52	8.3
12.....	4N					4	2	5	4	5	4	1	1																						22	9.5
13.....	4N					1		5	7	2	4																								26	7.3
14.....	4N					3	5	6	5	5	3	4	4																						30	7.9
15.....	4N					1	8	1	2	6	3	4	4	1	1	2																			27	10.9

<sup>1</sup> Plants 8-15 are from the same  $F_2$  population.

TABLE 2.—Number of microspores in "tetrads" of  $F_2$  asynaptic plants from *Gossypium hirsutum*  $\times$  *G. barbadense*

Plant No. <sup>1</sup>	Chromosome number	Number of microspores																		Number of "tetrads"	Average
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1 <sup>1</sup> .....	4N	1	1	...	12	18	42	23	35	10	8	4	2	1	...	...	...	...	...	157	6.9
1.....	4N	...	1	1	28	17	36	15	28	1	4	...	...	...	...	...	...	...	...	131	6.1
1.....	4N	...	...	...	16	12	54	58	36	12	3	...	...	...	...	...	...	...	...	191	6.7
1.....	4N	...	...	1	11	18	57	31	15	6	...	...	...	...	...	...	...	...	...	139	6.3
1 treated.....	8N	...	...	...	2	4	34	45	60	53	61	26	15	4	2	1	...	...	...	308	8.7
1 treated.....	8N	...	1	...	2	5	21	23	35	42	35	23	20	22	8	1	1	...	...	238	9.4
2.....	4N	...	...	...	11	19	46	37	47	32	15	9	5	...	...	...	...	...	...	221	8.1
2.....	4N	...	...	...	21	40	95	106	81	33	16	4	3	2	...	...	...	...	...	401	7.0
2 treated.....	8N	...	...	...	1	...	...	6	15	24	43	40	23	30	10	8	3	1	1	203	11.6
2 treated.....	8N	...	2	...	2	1	...	12	30	52	57	73	61	44	37	10	10	2	1	369	10.0
3.....	4N	13	62	40	714	183	162	37	10	...	...	...	...	...	...	...	...	...	...	1,221	4.4
3.....	4N	1	105	16	127	48	34	9	...	...	...	...	...	...	...	...	...	...	...	341	3.8
4.....	4N	1	24	13	34	21	34	48	19	5	2	2	...	...	...	...	...	...	...	203	5.5
5.....	4N	...	...	...	19	25	79	50	37	25	15	5	...	...	...	...	...	...	...	255	6.9
6.....	4N	...	...	...	4	8	29	32	38	33	30	...	...	...	...	...	...	...	...	174	7.8
7.....	4N	...	...	4	28	59	166	146	113	59	28	7	1	...	...	...	...	...	...	611	7.0
8 <sup>2</sup> .....	4N	...	8	4	77	24	63	14	9	...	...	...	...	...	...	...	...	...	...	200	5.1
9.....	4N	...	3	4	30	29	45	19	6	2	2	...	...	...	...	...	...	...	...	140	5.5
10.....	4N	...	...	2	12	18	57	38	48	17	8	...	...	...	...	...	...	...	...	200	6.8
11.....	4N	...	...	...	1	2	8	5	26	21	25	24	16	9	...	...	...	...	...	137	9.6
11.....	4N	...	...	...	13	19	36	39	24	13	5	3	...	...	...	...	...	...	...	153	6.7
12.....	4N	...	3	...	275	81	112	17	5	...	...	...	...	...	...	...	...	...	...	493	4.7
13.....	4N	...	...	...	7	15	42	43	40	32	18	3	...	...	...	...	...	...	...	200	7.4
14.....	4N	...	10	21	95	28	20	4	1	...	...	...	...	...	...	...	...	...	...	179	4.2
15.....	4N	...	3	4	29	21	69	40	23	8	2	...	1	...	...	...	...	...	...	200	6.1

<sup>1</sup> Plants with same plant number and chromosome number represent collections made from same plant on different dates.

<sup>2</sup> Plants 8-15 are from same  $F_2$  population

The pachytene chromosomes of *Gossypium* are unfavorable for study, but the observations that were made indicated that most of the chromosomes were paired at pachytene, as reported in asynaptic *Zea* (2) and *Pisum* (9).

The chromosome number of two asynaptic plants was doubled by colchicine treatment of grafts made from these plants. In meiosis 46 percent of the chromosomes of the treated plants paired as compared with 34 percent in the original asynaptic plants. Since environment is known to influence chromosome pairing and the material was collected at different times, the data do not justify the conclusion that doubling the chromosome number increased the amount of pairing. The range in the number of paired chromosomes in the asynaptic plants with double the original number of chromosomes was 15 to 33 (table 1 and fig. 3). The number of microspores in the "tetrads" reached as high as 18 (table 2). A few trivalents and quadrivalents were found in asynaptic plants with double the original number of chromosomes.

#### GENETICS OF ASYNAPTIC GOSSYPIUM PLANTS

An  $F_2$  progeny of *Gossypium hirsutum* (Stoneville 5)  $\times$  *G. barbadense* (Seabrook) gave 125 fertile plants and 8 asynaptic plants (table 3). A cross between an upland variety, Coker 100, and a sea-island strain, which was probably Bleakhall, gave 49 fertile and 5 sterile plants. A cross reported by Kearney (8) of *G. hirsutum* (Holden)  $\times$  *G. barbadense* (Pima) gave 200 fertile and 15 sterile plants. Kearney's description

leaves no doubt that the sterile plants he found were the asynaptic type. Of 22  $F_3$  progenies which he obtained from fertile  $F_2$  plants, 9 contained from 3 to 38 percent of completely sterile plants. Since the  $F_2$  ratio shows the asynaptic plants to be the result of two genes, both of which must be homozygous recessive, some  $F_3$  progenies would be expected to segregate 3 fertile to 1 sterile, and others would be expected to segregate 15 fertile to 1 sterile. In the  $F_3$  53.3 percent or 12 of 22 progenies would be expected to have sterile segregates. The range of 3 to 38 percent of sterile plants in  $F_3$  progenies would be expected in small  $F_3$  populations.

No asynaptic plants were found in an  $F_2$  population of over 500 of *G. hirsutum* (Half and Half)  $\times$  *G. barbadense* (Seabrook), in a population of over 100 of *G. hirsutum* (Half and Half)  $\times$  *G. barbadense* (Pima), or in a population of about 125 of *G. hirsutum* (Acala 8)  $\times$  *G. barbadense* (Seabrook).



FIGURE 3.—First meiotic metaphase of sterile plant with double the original number of chromosomes from  $F_2$  progeny of *Gossypium hirsutum*  $\times$  *G. barbadense* showing that doubling the chromosome number failed to restore normal pairing of the chromosomes.

#### DISCUSSION

The  $F_2$  ratio of 15 to 1 shows that the asynaptic *Gossypium* plants are the result of two pairs of recessive genes, one of which was shown to be homozygous in certain strains from three varieties of *G. hirsutum* and the other in two varieties of *G. barbadense*. Prof. G. W. Beadle has suggested to the authors that the situation might possibly be due to the same gene occurring homozygous in each of the two sets of chromosomes. Strains of two varieties of *G. hirsutum* (Half and Half and Acala 8) did not have the recessive gene. The mean difference in the number of bivalents and number of microspores in the "tetrads" of different asynaptic segregates collected the same day from the same population is evidence that modifying factors are present. Similar modifying factors were found by Beadle (1) in asynaptic *Zea* plants.

TABLE 3.—Normal and asynaptic plants in  $F_2$  populations of *Gossypium hirsutum*  $\times$  *G. barbadense*

Cross	Normal	Asynaptic	<i>D</i>
			<i>P. E.</i>
<i>G. hirsutum</i> (Stoneville 5) $\times$ <i>G. barbadense</i> (Seabrook).....	125	8	<1.0
<i>G. hirsutum</i> (Coker 100) $\times$ <i>G. barbadense</i> (Bleakhall?).....	49	5	1.3
<i>G. hirsutum</i> (Holden) $\times$ <i>G. barbadense</i> (Pima) <sup>1</sup> .....	200	15	<1.0
Total .....	374	28	<1.0

<sup>1</sup> Data from Keraney (8).

The percentage of the chromosomes pairing in the original asynaptic plants and in the plants with double the original number of chromosomes was approximately equal. The chromosomes failed to pair in the original  $F_2$  plants because of a gene combination. In doubling the chromosome number, the physiological effect of the genes in question was not changed, so pairing conditions remained the same. This fact had been noted by Dobzhansky (5) from studies of occasional polyploid cells in  $F_1$  hybrids between the A and B races of *Drosophila pseudoobscura*.

A statement by Dobzhansky concerning a sterile hybrid and its fertile polyploid finds no support in *Gossypium*. He states (5, p. 326):

\* \* \* One cannot, however, exclude the possibility that the chromosome pairing in the diploid is suppressed by the hybrid genetic constitution, but that the doubling of the chromosome complement entails a physiological change which removes the hindrance to bivalent formation.

If doubling the chromosome number resulted in a physiological reaction allowing the chromosomes to pair, it is probable that many of the chromosomes would form multivalents which would result in unstable polyploids. In all examples of polyploids in which chromosome pairing became normal, or nearly so, following the doubling of the chromosome number in a hybrid with no pairing or with pairing much reduced from normal, it is believed that the failure of pairing in the initial hybrid was the result of structural and genetic differences between the chromosomes, rather than the action of genes to prevent chromosome pairing. Evidence has been given (3) that structural differences exist between all the chromosomes in certain *Gossypium* hybrids that yielded highly fertile polyploids.

In asynaptic *Zea* plants Beadle (2) found an occasional attenuated bivalent and in some cells fragments. Koller (9) found attenuated bivalents in asynaptic *Pisum*, but he had evidence to show that the plant was heterozygous for an inversion, for no bridges were found in other asynaptic *Pisum* plants. In the asynaptic *Gossypium* no evidence was found that the attenuated chromosomes are the result of structural differences between chromosomes. It may be possible that the upset in meiosis that resulted in asynapsis could also at times cause attenuated bivalents. That these are not identical with bridges due to structural differences is evidenced by normal pairing in the  $F_1$ . Furthermore, doubling of the chromosome number in hybrids where pairing was absent or incomplete because of structural differences resulted in nearly normal pairing (3, 5), whereas doubling the number



of chromosomes in asynaptic plants produced little or no change in percentage of paired chromosomes.

It has been suggested that regular formation of bivalents, rather than multivalents, and normal fertility might be produced in autopolyploids by inducing mutations which reduce chiasma frequency. That this idea has no promise, at least in *Gossypium*, is shown by the autopolyploids from the asynaptic plants, for with only 46 percent of the chromosomes paired some trivalents and quadrivalents were present.

#### SUMMARY

In an  $F_2$  progeny of *Gossypium hirsutum*  $\times$  *G. barbadense* (American upland  $\times$  sea-island cotton), sterile asynaptic plants were found in a ratio of 15 fertile to 1 sterile. At first metaphase the fertile plants had the normal 26 pairs of chromosomes while the asynaptic plants averaged 6 to 12 pairs of chromosomes. Doubling the chromosome number in the sterile asynaptic plants failed to restore normal chromosome pairing and fertility.

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# THE INFLUENCE OF FEEDING LOW-NITROGEN RATIOS ON THE RELIABILITY OF BIOLOGICAL VALUES<sup>1</sup>

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## INTRODUCTION

For the past several years nitrogen balance experiments with lambs have been conducted at this station to study the quality of protein furnished by common feedstuffs (10, 11, 20, 21).<sup>3</sup> The nutritive value of the protein has been expressed as the percentage of total nitrogen stored, the percentage of digested nitrogen stored, and the biological value. In the later work the biological values reported are termed "estimated biological values," since average values for endogenous and metabolic nitrogen as determined in earlier experiments with lambs were used in the calculations.

The usual method of determining biological values of protein involves feeding a nitrogen-free or low-nitrogen ration during the experiment in order to measure the endogenous nitrogen losses in the urine and the metabolic nitrogen losses in the feces (12). Because of the difficulty in inducing experimental animals to consume sufficient amounts of either a nitrogen-free ration or a ration very low in nitrogen (8), certain investigators have added small amounts of some protein of high quality, such as egg protein. According to data reported by Mitchell and Carmen (14), the nitrogen excretions of rats fed a ration containing 0.6 to 0.75 percent of whole-egg nitrogen (3.75 to 4.69 percent protein) were practically the same as when they were fed a ration nearly nitrogen-free.

The time required by animals fed a low-nitrogen or nitrogen-free ration to reach an endogenous level of nitrogen losses is not definitely established. Smuts reports the time required by various animals to reach the endogenous level to be as follows: Mice, 5 days; guinea pigs, 8 days; rabbits, 15 days; and pigs, 20 days (17). Mitchell (12) and Chick and Roscoe (5), as well as others, have commonly used periods of 3 to 5 days for measuring the endogenous losses for rats. McCollum (6) and Mitchell and Hamilton (15) have used 10 to 15 days for swine. Turk et al. (20) and Sotola (19) have used a 10-day standardizing period for sheep. Martin and Robison report that for human subjects 5 to 7 days are required (7).

Ashworth and Brody (1, 2, 3), found that the minimum urinary nitrogen levels for rats were obtained at any time between 4 and 28 days on a nitrogen-free diet. Rats which had been previously fed a low-protein ration required less time to reach the endogenous level than rats previously fed a high-protein ration. Chick, Hutchinson, and Jackson (4) report that the excretion of endogenous nitrogen in the

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<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 450.

urine tends to diminish with the length of time that a rat is fed a diet devoid of nitrogen, although a period of 2 to 3 days is long enough to include the initial rapid fall in nitrogen excretion. They consider that a collection period of 4 days following a preliminary period of 2 to 3 days gives a fairly good estimate of the endogenous nitrogen in the urine, although the value may be regarded as a somewhat arbitrary quantity. Mitchell et al. (13) concluded that the level of endogenous nitrogen excretion as measured by their procedure possesses a definite biological significance and that the biological values determined possess an absolute as well as a relative significance.

In work at the Cornell station (9, 21), data have been obtained which might indicate that lambs store a higher percentage of the protein in an experimental ration following the feeding of a low-nitrogen ration. Considerable time may be required by lambs to reach a more or less stable level of protein utilization after such feeding and a 10-day preliminary period may not be of sufficient length.

Various problems have arisen regarding the accurate determination of the metabolic nitrogen fraction of the feces (16). The most common manner of expressing the metabolic nitrogen loss is in terms of dry-matter intake. Although body weight and possibly other factors may have some influence, dry-matter intake seems to be the predominant factor. However, it has been noted that when the experimental animals consumed but small amounts of the low-nitrogen rations, the amount of metabolic nitrogen excreted per unit of dry-matter intake was higher.

#### OBJECT OF EXPERIMENTS

The series of experiments with lambs reported in this paper were planned to obtain information on the following points:

- (1) The length of time required by lambs fed a low-nitrogen ration to reach their endogenous nitrogen level.
- (2) The effect on feed consumption, on losses in body weight, and on nitrogen excretion of adding small amounts of dried skim milk or of corn-gluten meal to low-nitrogen rations.
- (3) The time required by lambs to reach a stable level of nitrogen utilization when fed rations containing approximately 10 percent or more protein following the feeding of a low-nitrogen ration.
- (4) The influence of a low-nitrogen ration on the accuracy of biological values as heretofore obtained with lambs as a measure of quality of protein.

#### EXPERIMENT 1

##### PROCEDURE

Continuous nitrogen balance data were obtained on two grade wether lambs of mutton type by the use of metabolism cages. These cages, as well as the methods used in conducting the balance experiment, have been described previously (20). The urine and feces were collected at 2-day intervals and analyzed for nitrogen by the standard Kjeldahl procedure. Previous to the experiment the lambs had been fed a ration containing approximately 10 percent protein. Each lamb was fed the three different rations shown in table 1.



TABLE 1.—*Percentage composition and average protein content of rations used in experiment 1*

Ingredients	Low-nitrogen ration	High-nitrogen ration	Nitrogen-poor ration
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Cellulose.....	10.5	9.0	9.0
Starch.....	28.0	11.0	24.2
Sugar.....	28.0	10.7	24.2
Wheat straw.....	25.0	25.0	25.0
Corn oil.....	4.5	3.0	3.0
Minerals <sup>1</sup> .....	4.0	3.0	3.0
Dried skim milk.....		11.6	11.6
Linseed meal.....		26.7	
Total.....	100.0	100.0	100.0
Average protein content (N×6.25).....	1.05	13.16	4.49

<sup>1</sup> The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

Changes between rations were made abruptly by substituting one ration for another at a morning feeding, and collection of excreta was begun the same day. The first ration, which was fed for a period of 34 days, was the low-nitrogen basal ration for lambs developed at this station. The second ration, a high-nitrogen ration, was fed for 20 days following the feeding of the low-nitrogen ration. The purpose of feeding this ration was to bring the lambs back to a good state of nutrition, as well as to study the utilization of nitrogen immediately following a period of low-nitrogen intake.

Finally, a nitrogen-poor ration was fed for 40 days. This ration differed from the low-nitrogen ration mainly in containing enough dried skim milk to furnish approximately an average of 4 percent of protein and to bring the protein content of the ration to an average of 4.49 percent. Dried skim milk was selected as a feed which should furnish protein of as high quality as any feed which would be feasible to use in rations for lambs. If lambs respond in a manner similar to that reported for rats by Mitchell and Carmen (14), the losses of nitrogen of lambs fed this amount of protein in the ration should have been essentially the same as when they were receiving a nitrogen-free ration. The high-nitrogen ration, fed in the second period, was the nitrogen-poor ration plus sufficient linseed meal to furnish approximately 10 percent protein.

#### RESULTS

The results of experiment 1 are presented in table 2 and in figures 1, 2, 3, and 4. The curves presented in these and later figures are calculated logarithmic curves. The dots represent the actual determinations for each lamb.

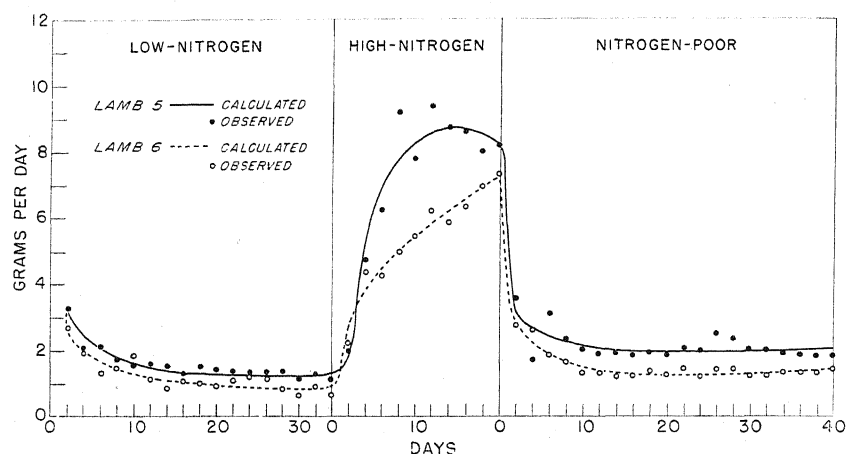


FIGURE 1.—Average daily losses of nitrogen in the urine of lambs fed a low-nitrogen ration, a high-nitrogen ration, and a nitrogen-poor ration.

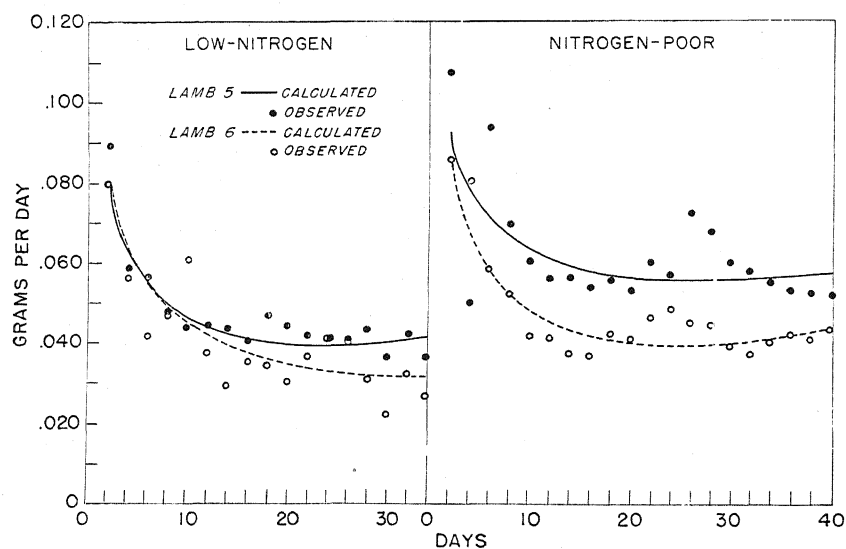


FIGURE 2.—Average daily losses of nitrogen per kilogram of body weight of the lambs when on the low-nitrogen ration and on the nitrogen-poor ration.

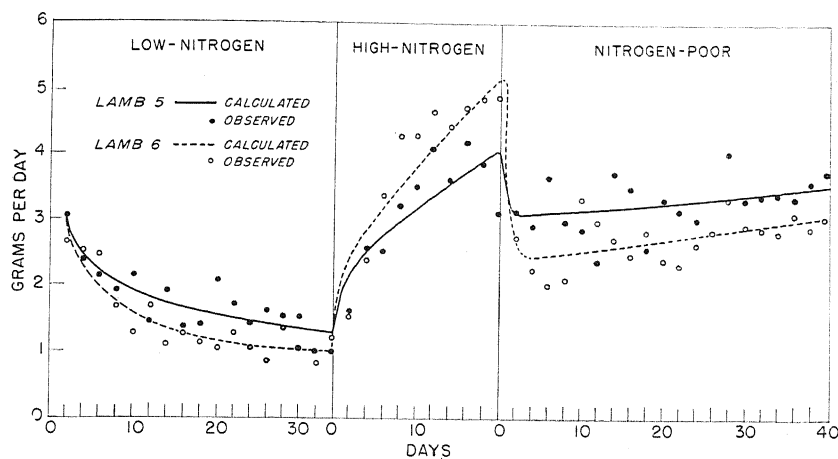


FIGURE 3.—Average daily losses of nitrogen in the feces of lambs fed a low-nitrogen ration, a high-nitrogen ration, and a nitrogen-poor ration.

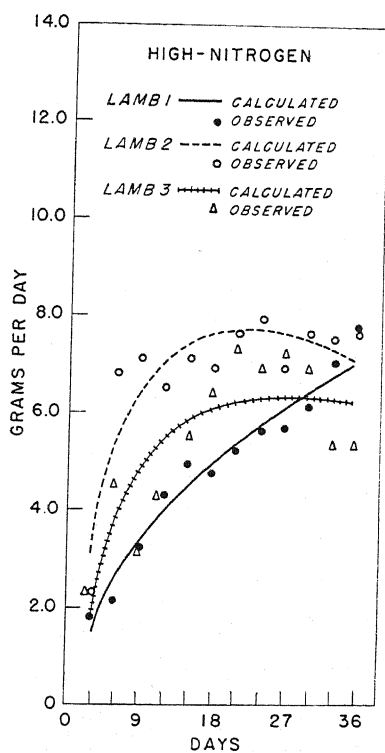


FIGURE 4.—Average daily losses of nitrogen in the feces per 100 gm. of dry-matter intake of lambs fed a low-nitrogen ration and a nitrogen-poor ration.

TABLE 2.—Nitrogen-balance data of experiment 1 by 2-day periods

Days on ration	Weight		Feed intake		Dry matter intake		Nitrogen intake		Nitrogen in urine		Nitrogen in feces	
	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6	Lamb 5	Lamb 6
	Kg.	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
2	36.4	33.3	1,200	1,200	1,119.0	1,119.0	2.10	2.10	6.52	5.32	6.10	5.33
4	36.2	32.4	1,200	900	1,119.0	839.2	2.10	1.58	4.06	3.78	4.72	5.00
6	35.9	31.8	1,200	1,200	1,119.0	1,119.0	2.10	2.10	4.06	2.65	4.29	4.93
8	35.7	31.1	1,200	400	1,119.0	373.0	2.10	.70	3.43	2.88	3.84	3.35
10	35.4	30.6	1,200	700	1,119.0	652.8	2.10	1.22	3.13	3.69	4.30	2.52
12	34.8	30.2	500	900	465.1	837.2	.84	1.52	3.10	2.26	2.90	3.37
14	34.3	29.5	850	650	790.7	604.6	1.43	1.10	2.99	1.73	3.83	2.20
16	32.1	29.0	600	700	558.1	651.1	1.01	1.18	2.59	2.06	2.73	2.59
18	31.7	28.8	700	600	651.1	558.1	1.18	1.01	2.98	2.00	2.80	2.25
20	31.8	28.6	1,000	525	930.2	488.4	1.68	.88	2.82	1.75	4.10	2.04
22	32.2	29.1	900	600	837.2	558.1	1.52	1.01	2.70	2.15	3.41	2.57
24	31.5	28.5	600	250	556.7	232.0	.97	.40	2.62	2.42	2.85	2.10
26	31.4	28.5	800	650	742.3	603.1	1.30	1.15	2.59	2.28	3.27	1.70
28	30.8	27.8	800	700	742.3	649.5	1.30	1.13	2.69	1.73	3.08	2.72
30	30.6	27.7	800	400	742.3	371.2	1.30	.65	2.23	1.26	3.04	2.06
32	30.1	27.2	250	325	232.0	301.6	.40	.53	2.54	1.75	2.01	1.67
34	29.6	27.1	600	567	556.7	526.1	.97	.92	2.15	1.45	1.97	2.43

HIGH-NITROGEN RATION												
2	29.8	27.4	750	750	698.8	698.8	15.79	15.79	3.87	4.43	3.20	3.06
4	30.5	28.1	1,100	1,100	1,025.0	1,025.0	23.16	23.16	9.28	8.66	5.11	5.26
6	30.4	28.7	1,225	1,225	1,141.5	1,141.5	25.79	25.79	12.36	8.50	5.02	6.75
8	31.3	29.4	1,325	1,325	1,234.6	1,234.6	27.89	27.89	18.34	9.90	6.41	8.54
10	31.5	30.0	1,550	1,550	1,444.3	1,444.3	32.63	32.63	15.54	10.98	6.99	8.52
12	31.8	30.7	1,600	1,600	1,490.9	1,490.9	33.68	33.68	18.74	12.36	8.17	9.30
14	31.3	30.1	1,600	1,600	1,490.9	1,490.9	33.68	33.68	17.40	11.68	7.19	7.86
16	32.8	31.6	1,600	1,600	1,490.9	1,490.9	33.68	33.68	17.22	12.64	8.33	8.40
18	32.7	32.0	1,600	1,600	1,490.9	1,490.9	33.68	33.68	15.97	13.94	7.68	9.67
20	32.5	32.1	1,600	1,600	1,490.9	1,490.9	33.68	33.68	16.38	14.62	6.22	9.74

NITROGEN-POOR RATION												
2	33.1	32.3	1,600	1,200	1,493.8	1,120.3	11.62	8.71	7.14	5.52	6.23	5.42
4	34.0	32.6	1,600	500	1,493.8	466.8	11.62	3.63	3.40	5.25	5.82	4.46
6	33.2	31.7	1,600	900	1,493.8	840.2	11.62	6.53	6.24	3.71	7.25	4.01
8	33.5	31.2	1,600	1,100	1,493.8	1,027.0	11.62	7.99	4.70	3.26	5.93	4.22
10	34.0	31.3	1,600	1,450	1,493.8	1,353.7	11.62	10.53	4.11	2.63	5.67	6.63
12	34.2	31.5	1,600	1,300	1,493.8	1,213.7	11.62	9.44	3.83	2.61	6.72	5.98
14	34.3	31.8	1,600	1,250	1,493.8	1,167.0	11.62	9.08	3.88	2.37	7.44	5.41
16	34.1	32.0	1,600	1,250	1,493.8	1,167.0	11.62	9.08	3.67	2.38	6.98	4.91
18	34.1	31.6	1,600	500	1,493.8	466.8	11.62	3.63	2.77	2.68	5.13	5.65
20	34.5	30.8	1,600	850	1,477.8	785.1	11.41	6.05	3.66	2.54	6.62	4.77
22	34.2	31.0	1,600	1,050	1,477.8	969.8	11.41	7.49	4.14	2.89	6.28	4.63
24	34.0	31.3	1,600	1,400	1,477.8	1,293.0	11.41	9.98	3.88	2.42	6.01	5.22
26	35.0	31.6	1,600	1,400	1,477.8	1,293.0	11.41	9.98	5.06	2.84	5.59	5.66
28	34.4	32.0	1,600	1,600	1,477.8	1,477.8	11.41	11.41	4.68	2.86	8.05	7.16
30	34.6	32.4	1,600	1,350	1,482.6	1,250.9	11.36	9.58	4.14	2.54	6.63	5.81
32	34.5	32.4	1,600	1,250	1,482.6	1,158.2	11.36	8.88	3.99	2.40	6.74	5.73
34	34.3	32.1	1,600	1,200	1,482.6	1,111.9	11.36	8.32	3.77	2.60	6.73	5.59
36	34.2	31.3	1,600	1,400	1,482.6	1,297.2	11.36	9.94	3.66	2.64	6.63	6.12
38	35.0	32.2	1,600	1,400	1,482.6	1,297.2	11.36	9.94	3.66	2.64	7.12	5.76
40	34.7	32.1	1,600	1,350	1,482.6	1,250.9	11.36	9.58	3.62	2.78	7.40	6.09

## FEED INTAKE

Each lamb was fed 600 gm. per day of the low-nitrogen ration at the start with the hope that at this rather low level of intake all the feed would be consumed. However, after a short time, neither lamb would consume that amount of feed. Lamb 5 tended to have a stronger appetite than lamb 6 throughout the period, although this lamb refused to consume 600 gm. of the low-nitrogen ration after the tenth day. After each lamb had refused to eat as much of the ration as planned, the ration was fed according to appetite.



The appetite of each lamb improved markedly when the high-nitrogen ration was fed. Both lambs made a regular increase in feed consumption until each consumed 800 gm. per day after the tenth day. This level of feeding was continued during the remainder of the period on this ration.

Lamb 5 continued to eat 800 gm. per day when fed the nitrogen-poor ration and his appetite remained good. The appetite of lamb 6 was somewhat irregular on this ration. He ate less than when fed the high-nitrogen ration, but considerably more than when fed the low-nitrogen ration.

#### BODY WEIGHT

Both lambs lost weight steadily during the period in which the low-nitrogen ration was fed. The losses were greater during the intervals of low-feed intake. However, at no time did the lambs maintain their weight when fed the low-nitrogen ration. Each lamb started to gain in weight immediately upon changing to the high-nitrogen ration, and continued making good gains throughout the period.

Lamb 5 continued to make slight gains in weight when fed the nitrogen-poor ration. This small gain amounted to approximately 1.5 kg. for the 40-day period. Probably because of lower feed consumption, lamb 6 just maintained his weight when fed this ration.

#### URINARY NITROGEN LOSSES

The urinary nitrogen losses declined abruptly during the first 10 days the lambs were fed the low-nitrogen ration, but after the first 10 or 12 days, the decline was slight (fig. 1).

The fact that the lambs excreted the largest daily amount of urinary nitrogen during the first 10 days of feeding may be explained largely by the residual effect of the former ration, which contained approximately 10 percent protein. The small decline in rate of excretion of urinary nitrogen following this initial drop may be due, in part, to the fact that the lambs were gradually losing in body weight. Also, the lambs may have become gradually more efficient in the utilization of the nitrogen until they reached a so-called endogenous level.

As would be expected, the urinary nitrogen losses increased when the lambs were fed the high-nitrogen ration. However, the amount excreted was relatively small at the start, showing that nitrogen was being retained in large amounts, with considerable of it undoubtedly remaining in the digestive tract. After approximately the tenth day, the nitrogen excretion in the urine did not change greatly. However, as indicated in figure 1, there was no positive evidence that the lambs had reached a fairly constant level of nitrogen excretion in the urine during this 20-day period.

The urinary nitrogen dropped immediately when the lambs were fed the nitrogen-poor ration. However, as shown in figure 1, it did not reach such low levels when the lambs were fed this ration containing 4.49 percent protein chiefly from dried skim milk as when they were fed a low-nitrogen ration containing 1.05 percent protein.

It would therefore be obviously incorrect to consider that the urinary nitrogen excretion on the nitrogen-poor ration represented solely endogenous nitrogen excretion. Without doubt some of the nitrogen excreted during this period was of feed origin.

As shown in figure 2, the losses of urinary nitrogen per kilogram of body weight follow the same trend as that of the total urinary nitrogen excretion. The greatest drop occurred during the first 10 days. After this drop there was no positive evidence that the values for endogenous nitrogen per kilogram of weight became progressively lower with time.

#### FECAL NITROGEN LOSSES

Fecal nitrogen losses were also greater when the low-nitrogen ration was first fed than after the first 6 or 8 days. This initial high excretion of nitrogen in the feces was undoubtedly due mainly to the previous higher level of feeding and higher nitrogen content of the ration. After this short time, the fecal nitrogen excretion was definitely correlated with the total feed intake on the low-nitrogen ration.

Another lag in fecal nitrogen excretion was observed when the lambs were first fed the high-nitrogen ration. As shown in figure 3, the nitrogen losses were appreciably smaller until after approximately the tenth day. Following this time the fecal nitrogen losses appeared to be mainly correlated with the feed or nitrogen intake.

The fecal nitrogen dropped to a lower level when the lambs were fed the nitrogen-poor ration instead of the high-nitrogen ration. However, considerably more nitrogen was excreted than when the low-nitrogen ration was fed. This level of nitrogen excretion remained more or less constant for each lamb throughout the feeding of the nitrogen-poor ration.

The values for metabolic nitrogen calculated by 2-day periods show a strong correlation between feed intake and fecal nitrogen losses (fig. 4). If the first few periods are omitted, the fecal nitrogen losses of the lambs on both the low-nitrogen and the nitrogen-poor rations appear to be governed by the level of feed intake rather than by the nitrogen content or by the length of time the lambs were fed the rations.

The nitrogen in the high-nitrogen ration was utilized with high efficiency. During the last 10 days of the high-nitrogen period of feeding, lamb 5 retained on an average 26.8 percent of the total nitrogen in the ration while lamb 6 retained an average of 34.6 percent. The percentage of total nitrogen retained by the lambs during the first 10 days of this period was even greater.

In a previous experiment at this station (9), in which there were no preliminary low-nitrogen periods, lambs fed a ration containing approximately 10 percent of protein furnished almost entirely by dried skim milk stored 19.8 percent of the nitrogen. Lambs fed a similar ration except that it contained linseed meal as the source of protein stored 22.7 percent of the total nitrogen. These lower values for nitrogen storage were obtained even though less protein was fed than in the experiment reported in this paper. Therefore these high values for nitrogen utilization were probably due largely to the influence of the previous period of low-nitrogen feeding.

## EXPERIMENT 2

### PROCEDURE

In the second experiment, continuous nitrogen balance data were obtained on four lambs. The methods used were similar to those

employed in experiment 1, except that the collection of the excreta was made at 3-day intervals instead of 2-day intervals, in order to lessen the cost.

Each of the four lambs was fed a ration containing slightly more than 10 percent protein during an initial period of 20 days. Lambs 1 and 2 were fed a ration in which most of the protein was furnished by soybean oil meal, while lambs 3 and 4 received a similar ration except that it contained corn-gluten meal. Nitrogen balances were determined on each lamb during the last 9 days of the period.

Following the above initial experimental period, each lamb was fed a low-nitrogen ration containing approximately 2.75 percent protein. The change in ration was made abruptly at a morning feeding and the nitrogen balances were continued without interruption.

The rations used in this period were the same as the low-nitrogen basal ration used during the first phase of experiment 1, except that some of the starch and sugar was replaced with dried skim milk or corn-gluten meal. These protein-rich feeds furnished approximately 1.2 percent protein in the rations, or nearly one-half the total protein, with wheat straw furnishing the remainder. Lambs 1 and 3 were fed the dried skim milk ration and lambs 2 and 4 were fed the corn-gluten meal ration.

The original plan was to obtain nitrogen balance data on each lamb during an experimental period of 51 days without change from the low-nitrogen ration. Unfortunately, this plan was actually followed on only one lamb, as explained in the discussion of results.

In the last phase of this experiment, each lamb was again fed the same ration as at the start. Collections were made and nitrogen balances were obtained on each lamb through a period of 39 days. The rations fed during experiment 2 are given in table 3.

TABLE 3.—Percentage composition, and average protein content of rations used in experiment 2

Ingredients	High-nitrogen rations		Low-nitrogen rations	
	Soybean oil meal	Corn-gluten meal	Dried skim milk	Corn-gluten meal
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Cellulose.....	9.0	9.0	10.5	10.5
Starch.....	17.78	18.31	26.32	26.83
Sugar.....	17.77	18.30	26.32	26.84
Wheat straw.....	25.0	25.0	25.0	25.0
Corn oil.....	3.0	3.0	4.0	4.0
Minerals <sup>1</sup> .....	3.0	3.0	4.0	4.0
Soybean oil meal.....	24.45			
Corn-gluten meal.....		23.39		2.73
Dried skim milk.....			3.86	
Total.....	100.00	100.00	100.00	100.00
Average protein content (N × 6.25).....	11.03	10.76	2.73	2.75

<sup>1</sup> The mineral mixture was made up of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

#### RESULTS

The results of experiment 2 are shown in table 4 and in figures 5 and 6.

TABLE 4.—Nitrogen balance data of experiment 2 by 3-day periods  
HIGH-NITROGEN RATION

Days on ration	Weight of lamb				Feed intake				Dry-matter intake				Nitrogen intake				Nitrogen in urine				Nitrogen in feces			
	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4
3	Kg.	Kg.	Kg.	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
6	31.2	33.6	34.2	34.2	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	43.68	43.68	43.68	44.40	20.01	20.01	17.38	27.69	13.47	14.37	12.99	11.71
9	32.2	32.4	33.7	33.7	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	43.64	43.64	43.64	44.40	23.80	23.80	28.22	26.37	14.31	14.68	15.90	18.43
12	32.0	33.9	34.3	33.9	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	43.64	43.64	43.64	44.16	27.26	27.26	21.81	26.42	15.64	17.11	12.32	14.30

## LOW-NITROGEN RATION

Days on ration	Weight of lamb				Feed intake				Dry-matter intake				Nitrogen intake				Nitrogen in urine				Nitrogen in feces			
	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Lamb 1	Lamb 2	Lamb 3	Lamb 4
3	Kg.	Kg.	Kg.	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
6	31.2	33.6	34.0	34.4	2,000	2,400	2,400	2,400	1,852.8	2,238.2	2,238.2	2,238.2	8.20	9.12	9.12	9.12	10.94	8.99	9.05	9.78	13.59	17.76	12.75	13.08
9	30.6	33.7	34.1	34.2	2,150	2,400	2,400	2,400	1,973	2,238.2	2,238.2	2,238.2	8.82	9.12	9.12	9.12	4.13	4.37	4.55	7.26	10.02	13.60	10.08	7.37
12	30.4	32.7	34.6	32.5	1,800	1,924	1,924	1,924	1,800	1,700	1,700	1,700	7.79	7.31	7.31	7.31	4.10	5.13	4.02	5.94	8.27	10.74	5.84	6.57
15	30.4	30.8	34.4	32.3	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	4.14	5.46	5.46	5.46	3.64	8.36	3.55	3.39	6.71	6.01	9.33	7.51
18	28.4	31.8	34.4	31.7	1,300	1,800	1,800	1,800	1,000	1,222.9	1,222.9	1,222.9	4.97	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
21	28.4	31.7	34.2	31.9	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
24	28.0	30.9	34.4	31.9	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
27	27.3	30.7	33.6	32.9	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
30	25.8	31.6	33.5	32.7	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
33	25.1	31.8	33.5	32.0	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
36	23.8	33.0	32.3	32.2	1,300	1,800	1,800	1,800	1,300	1,222.9	1,222.9	1,222.9	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
39	33.0	32.9	32.3	32.5	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
42	33.0	32.9	32.3	32.5	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
45	32.1	31.8	32.3	32.5	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
48	33.4	31.6	32.3	32.5	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65
51	32.5	31.0	32.7	32.7	1,800	1,800	1,800	1,800	1,800	1,800	1,800	1,800	5.38	30.78	30.78	30.78	4.32	4.85	4.15	3.18	6.50	7.49	8.28	6.65



## HIGH-NITROGEN RATION

2	22.5	33.1	30.4	33.0	650	2 900 <sup>1</sup>	1,850	2 000	600.0	1,838.8	1,710.5	1,819.2	11.82	36.22	34.04	36.80	5.26	6.94	4.76	7.50	3.29	6.36	6.03	7.00
6	23.8	33.5	31.5	33.2	1,600	2 100	1,350	2 200	1,478.8	1,920.7	1,218.2	2,034.1	28.09	38.01	24.84	40.48	6.29	20.38	13.51	16.50	12.25	12.81	8.12	9.69
9	26.7	33.2	31.7	33.7	1,900	2 400	1,000	2 400	1,752.7	2,206.6	1,921.6	2,218.0	34.35	43.47	18.40	44.16	9.60	21.40	9.18	18.70	9.94	11.12	6.77	9.69
12	28.0	33.9	31.3	34.3	2,400	2 400	2 100	2 400	2,015.2	2,218.6	1,941.7	2,218.0	43.64	41.04	38.64	38.64	14.82	19.41	12.89	19.75	14.52	11.67	7.88	10.63
15	29.1	35.2	32.6	35.1	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	21.38	16.47	20.82	13.38	11.57	12.57	10.06
18	29.9	35.2	34.9	35.3	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	20.76	19.25	20.92	12.23	11.95	12.04	14.87
21	30.3	35.8	36.0	35.3	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	22.80	21.91	22.29	13.05	12.75	13.31	15.78
24	30.4	35.6	35.6	34.2	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	23.64	20.80	22.59	14.17	13.85	12.77	15.20
27	30.5	36.0	36.6	33.4	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	24.75	21.08	23.95	13.60	12.30	14.40	16.52
30	30.7	36.1	37.2	33.6	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,216.2	2,216.2	43.47	41.04	38.64	38.64	14.82	25.66	20.67	20.06	12.71	10.21	15.13	10.38
33	31.7	35.6	36.9	33.4	2,400	2 400	2 400	2 400	2,206.6	2,218.6	2,231.5	2,231.5	41.04	41.10	38.83	38.83	21.08	22.38	15.04	15.31	14.69	15.35	15.02	12.63
36	32.7	26.5	37.8	34.8	2 400	2 400	2 400	2 400	2,206.6	2,218.6	2,231.5	2,231.5	41.04	41.10	38.83	38.83	23.39	22.80	16.05	25.92	15.63	11.05	13.31	14.16

<sup>1</sup> Lambs 2 and 4 were fed the high-nitrogen corn-gluten meal ration for 18 days, beginning with the eighteenth day.

All lambs consumed the same amount of the high-nitrogen rations during the initial period. Lambs 3 and 4, which were fed the corn-gluten meal ration, excreted slightly more nitrogen in the urine than the other two lambs, which were fed the soybean oil meal ration. However, the difference was significant only in the case of lamb 4. This lamb proved to be less efficient than the other lambs throughout the entire experiment.

As stated previously, the rations fed each of the four lambs were changed abruptly to either the dried skim milk low-nitrogen ration or the corn-gluten meal low-nitrogen ration. Lamb 1 was fed the dried skim milk ration for a period of 36 days. This lamb refused to eat well, especially after the twenty-first day. Because of the low state of nutrition of this lamb and its decided lack of thriftiness,

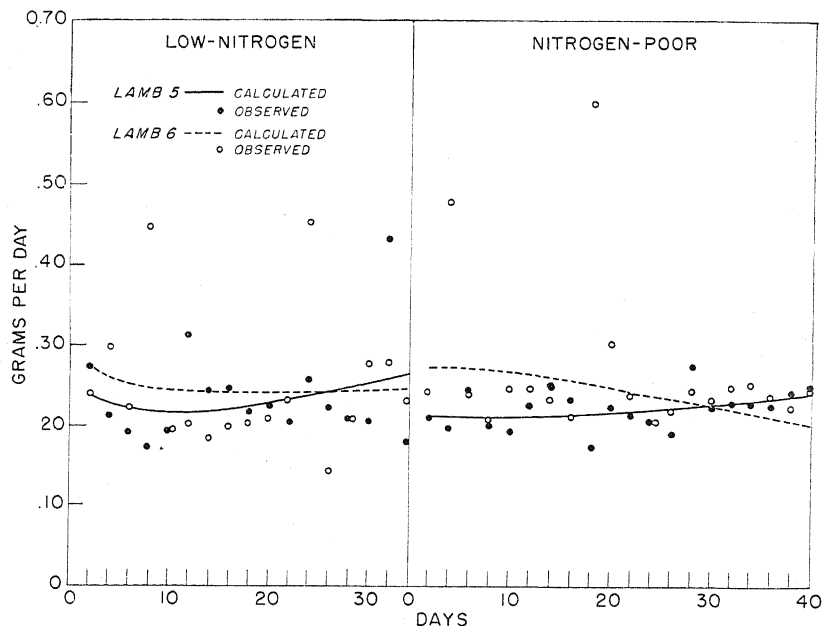


FIGURE 5.—Average daily losses of nitrogen in the urine of lambs when fed the high-nitrogen ration.

this ration was discontinued and the high-nitrogen ration of the next phase of the experiment was fed.

Lambs 2 and 4, which should have been fed the low-nitrogen corn-gluten meal ration throughout this period, were by error fed the high-nitrogen corn-gluten meal ration from the eighteenth to the thirty-sixth day. Immediately following this period during which the high-nitrogen ration was fed, the lambs were again fed the former low-nitrogen ration. Lamb 3 was fed the dried skim milk low-nitrogen ration for 51 days.

The lambs in this experiment reacted much as the lambs in experiment 1 reacted when the low-nitrogen basal ration was substituted for a high-nitrogen ration. The amount of feed the lambs consumed was less by the ninth day or earlier, and they lost weight. These data indicate that the declines in feed consumption and body weight were

not so severe as in experiment 1 when lambs were fed the low-nitrogen basal ration, but were somewhat greater than when the lambs were fed the nitrogen-poor ration containing approximately 4 percent protein.

The excretions of urinary nitrogen decreased rapidly for each lamb until approximately the ninth or twelfth day. After that time no significant trend was evident in the urinary nitrogen excretion by the two lambs which were continued on the low-nitrogen rations. In the case of lambs 2 and 4, a considerable increase in the urinary

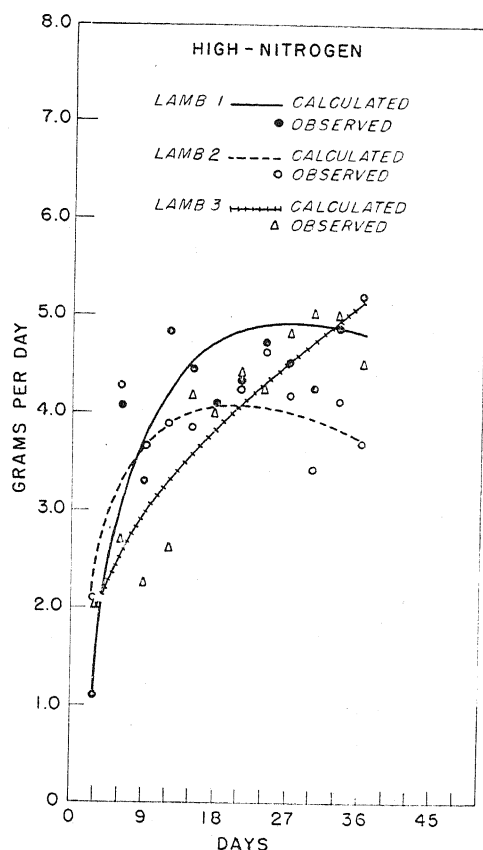


FIGURE 6.—Average daily losses of nitrogen in the feces of lambs when fed the high-nitrogen ration.

nitrogen losses appeared in the first 3-day period following the feeding of the high-nitrogen ration. When the feeding of this ration was discontinued, the lambs again excreted at the low levels of urinary nitrogen. Neither lamb excreted as much urinary nitrogen per day during this 18-day period as during the initial period when the high-nitrogen rations were fed. However, the feed intake was also lower, which would account for much of the difference. The average percentage of nitrogen intake that was excreted in the urine was about the same for the two lambs during the last 9 days as during the initial 9-day collection period.

The fecal nitrogen excretions of the lambs likewise reached a fairly stable level by the ninth to the twelfth day of the low-nitrogen period. From that time on the fecal nitrogen losses appeared to be more closely correlated with feed intake than with the length of time the lambs were fed the low-nitrogen ration.

All lambs regained their appetites fairly promptly when changed to the high-nitrogen ration. The feed was held at the same level that each lamb had consumed of the ration during the first phase of the experiment. All but lamb 4 consumed this amount of feed regularly until the end of the experiment.

Good gains were made by all the lambs when fed the high-nitrogen ration. Lamb 4, however, failed to gain as much as the other lambs, since this lamb went off feed for a few days during the period.

The urinary nitrogen excretions rose when the changes in rations were made. As shown in figure 5, some time was required for the lambs to become adjusted to the higher feed and nitrogen intakes. The urinary nitrogen excretions appeared to become more uniform for each lamb by the time the lambs had consumed the desired amounts of the rations. However, the lambs excreted somewhat less nitrogen in the urine for some time, even after allowing for the lower feed consumption and the lag in urinary nitrogen losses. In order to study this problem further and to remove the effect of slight variations in the nitrogen intake by the lambs, the urinary nitrogen losses were converted into the percentage of nitrogen intake that was excreted in the urine. Only the data for lambs 1, 2, and 3, which completed the 36-day period without going off feed, were considered.

The percentage of the nitrogen intake that was excreted in the urine was extremely variable during the first 9 days. However, as might be expected, the lambs in each case excreted a lower percentage of the nitrogen intake during this 9-day period than during any of the other three 9-day periods. The data for each lamb indicated that a lower percentage of the nitrogen intake was excreted in the urine during the second 9-day period (tenth to eighteenth day, inclusive) than during the third 9-day period (nineteenth to twenty-seventh day, inclusive). Treatment of these data statistically by the analysis of variance method (18) shows that this difference is highly significant. These results would indicate that a 9- or 10-day intervening period following the low-nitrogen period is not enough time to enable lambs to regain their adequate consumption and reach a normal state of nutrition. Therefore, nitrogen-balance data obtained under such conditions would result in values for protein utilization that would be too high.

A comparison of the percentage of nitrogen intake excreted in the urine by the lambs during the third 9-day period (nineteenth to twenty-seventh day, inclusive) with that excreted during the fourth 9-day period (twenty-eighth to thirty-sixth day, inclusive) does not show a significant difference on the average. In the case of lamb 1, there was a marked difference, as the nitrogen losses were appreciably greater during the fourth period than during the third period. There was practically no difference in the data for the two periods for lamb 2; for lamb 3, the trend was somewhat reversed.

As shown in table 4, lamb 1 did not eat the low-nitrogen ration as well as the other lambs. Consequently, he lost more weight and entered the high-nitrogen period in a much poorer state of nutri-



tion. Lamb 2 was undoubtedly in the best state of nutrition, as shown by a lower loss of weight during the second phase of the experiment. It is of interest to note that lamb 1 excreted the least urinary nitrogen of the three lambs throughout the high-nitrogen period and apparently did not reach a stable level of urinary nitrogen losses during the course of the experiment. On the other hand, lambs 2 and 3 apparently reached a more or less stable rate of urinary nitrogen excretion sometime during the nineteenth to the twenty-seventh-day period.

With the exception of lamb 2, the lambs failed, in general, to excrete as much nitrogen daily in the urine as they did during the first phase of the experiment when eating the same amounts of the same rations. These data indicate that the lambs were somewhat more efficient in their use of nitrogen as a result of having been previously fed rations low in nitrogen.

The fecal nitrogen losses during this phase of the experiment appeared to be well correlated with the feed intake after the first few days (fig. 6). No definite conclusions can be drawn concerning the relative efficiency with which the lambs digested or utilized the protein of soybean oil meal and corn-gluten meal. The data on the nitrogen excretion for lamb 4 are irregular because of irregular feed intake. While lamb 3, which was fed the corn-gluten meal ration, retained slightly less nitrogen than lambs 1 and 2, which were fed the soybean oil meal ration, the difference could not be considered significant.

### EXPERIMENT 3

#### OBJECT

The object of experiment 3 was to determine the effect that the feeding of a low-nitrogen ration may have on the efficiency with which lambs later retain nitrogen.

#### PROCEDURE

Nitrogen balance trials with eight growing wether lambs were conducted to study this problem. Four different test rations and a low-nitrogen basal ration were fed. One group of four lambs was used in a series of trials in which two hay rations were compared; the remaining four lambs were fed two rations having either corn-gluten meal or soybean oil meal as nearly the entire source of nitrogen.

These particular rations were used in order to supplement data previously obtained at this station (10, 21). In the earlier experiments, the data indicated a slight superiority of the nitrogen in the one-third alfalfa and two-thirds timothy ration as compared with the alfalfa ration. Also, the nitrogen in the soybean oil meal ration was found to be slightly superior to that furnished by the corn-gluten meal ration.

At the beginning of this experiment each lamb was fed one of the four rations in place of his usual barn ration. Ten days after each lamb was safely on a good level of feeding, nitrogen balance trials were begun.

After each group of four lambs had been fed each of the two rations, the low-nitrogen ration was fed. This change of rations was made abruptly by changing the rations at a morning feeding. On the eleventh day of feeding the low-nitrogen ration the collections for nitrogen balances were started.

Following the low-nitrogen period, each lamb was fed the same experimental ration that he received at the start of the experiment. Again the change in feeding was made abruptly, and collection of excreta was begun on the eleventh day following the change. The order of feeding was also the same as in the first part of the experiment. The rations are shown in table 5.

TABLE 5.—Percentage composition, and average protein content of rations used in experiment 3

Ingredients	Soybean oil meal ration	Corn-gluten meal ration	Alfalfa ration	$\frac{1}{3}$ alfalfa- $\frac{2}{3}$ timothy ration	Low-nitrogen ration
	Percent	Percent	Percent	Percent	Percent
Wheat straw	25.0	25.0			25.0
Cellulose	9.0	9.0			10.5
Alfalfa			50.0	16.7	
Timothy				33.3	
Corn			34.0	44.4	
Soybean oil meal	24.3			4.2	
Corn-gluten meal		23.04			
Starch	18.0	18.96	14.0		28.0
Sugar	17.7	18.00			28.0
Corn oil	3.0	3.00	1.0		4.5
Minerals <sup>1</sup>	3.0	3.00	1.0	1.4	4.0
Total	100.0	100.00	100.0	100.0	100.0
Average protein content (N $\times$ 6.25)	11.4	11.4	9.5	9.7	1.05

<sup>1</sup> The mineral mixture was composed of 40 percent ground limestone, 40 percent steamed bonemeal, and 20 percent salt.

#### RESULTS

The results of experiment 3 are shown in table 6.

TABLE 6.—Summary of digestibility and utilization of the nitrogen in the rations fed in experiment 3

Item	Before low-nitrogen period					After low-nitrogen period				
	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Average	Lamb 1	Lamb 2	Lamb 3	Lamb 4	Average
Alfalfa ration:										
Apparent digestibility	Pct. 61.7	Pct. 66.8	Pct. 66.0	Pct. 65.5	Pct. 65.0	Pct. 55.7	Pct. 67.0	Pct. 56.6	Pct. 65.3	Pct. 61.2
Total nitrogen retained	29.0	16.0	33.3	18.4	24.2	16.4	20.7	29.5	27.7	23.6
Digestible nitrogen retained	47.0	24.1	50.4	28.0	37.4	29.5	30.9	52.0	42.5	38.7
Biological value	74	58	75	60	67	67	63	79	71	70
Alfalfa $\frac{1}{3}$ , timothy $\frac{2}{3}$ :										
Apparent digestibility	65.0	69.6	64.8	67.9	66.8	62.1	69.8	64.8	60.1	64.2
Total nitrogen retained	19.2	30.9	26.2	18.4	23.7	32.7	29.0	31.3	33.4	31.6
Digestible nitrogen retained	29.6	44.4	40.4	27.0	35.4	52.7	41.6	48.3	55.5	49.5
Biological value	62	69	68	58	64	79	68	75	81	76
	Lamb 5	Lamb 6	Lamb 7	Lamb 8		Lamb 5	Lamb 6	Lamb 7	Lamb 8	
Soybean oil meal:										
Apparent digestibility	72.7	71.7	72.1	72.0	72.1	74.6	72.2	65.0	70.5	70.6
Total nitrogen retained	28.5	18.0	29.3	20.1	24.0	28.0	27.6	31.1	24.3	27.8
Digestible nitrogen retained	39.2	25.2	40.7	28.0	33.3	37.5	38.3	47.8	34.5	39.5
Biological value	70	52	62	55	60	68	62	69	61	65
Corn-gluten meal:										
Apparent digestibility	71.9	73.0	69.9	70.6	71.4	71.1	69.5	64.5	68.6	68.4
Total nitrogen retained	25.0	32.9	26.0	20.1	26.0	27.7	29.7	31.9	24.7	28.5
Digestible nitrogen retained	34.8	45.1	37.2	28.4	36.4	39.0	42.7	49.4	36.0	41.8
Biological value	68	67	61	56	63	72	66	72	63	68

The nitrogen contained in the alfalfa ration was digested somewhat less efficiently than the nitrogen contained in the one-third alfalfa two-thirds timothy ration. The data on digestibility obtained previous to the low-nitrogen period check unusually well with data obtained in the earlier experiment in which the alfalfa ration was found to have an apparent digestibility of  $64.5 \pm 0.71$  and the one-third alfalfa two-thirds timothy ration an apparent digestibility of  $66.7 \pm 0.84$ . There was no difference in the digestibility of the nitrogen in the soybean oil meal and in the corn-gluten meal rations, although each showed a higher digestibility than either of the two hay rations. In nine comparisons the digestibility after the low-nitrogen period was lower by as much as 1 percent than the digestibility before the low-nitrogen period. In six comparisons there was no difference, and in only one case was the digestibility higher after the low-nitrogen period. A treatment of these data statistically, by Student's method (18), shows a high mathematical significance.

The percentage of the total nitrogen retained by the lambs averaged higher following the low-nitrogen period. There were 5 exceptions among the 16 comparisons. A similar statistical treatment of these data shows a mathematically significant difference with odds of 60:1 that the higher values obtained following the low-nitrogen period were not due to chance alone.

There was a somewhat greater difference in the percentage of digested nitrogen retained before and after the low-nitrogen period than in the percentage of total nitrogen stored. This greater difference may be explained by the lower digestibility of the nitrogen following the low-nitrogen period. The odds were approximately 300:1 that the greater retention of digested nitrogen following the low-nitrogen period was not due to sampling error. Also, the biological values computed from these data averaged higher following the low-nitrogen period. The odds were approximately 300:1 and therefore these differences were mathematically significant.

This experiment was so planned that the nitrogen balance trials for two lambs fed each ration were conducted from the eleventh to the twentieth day, inclusive, following the low-nitrogen period, while the trials for the other two lambs fed the same ration were conducted from the twenty-first to the thirtieth day, inclusive. Thus, a comparison was possible on the utilization of the nitrogen between lambs at these two intervals of time following the low-nitrogen period. However, it should be noted that these data are not strictly comparable since different lambs were used in the two periods. There were eight comparisons between these two periods available for study.

It is of interest to note that the nitrogen losses in the urine for those lambs for which data were obtained during the eleventh- to twentieth-day period averaged lower than for those which were on the collection period during the twenty-first- to thirtieth-day period. The average apparent digestibility of the nitrogen in the rations for the earlier period was also lower than for the later period. On the other hand, there was practically no difference in the percentage of total nitrogen stored, the percentage of digested nitrogen stored, or the biological values.

The above data agree well with those obtained in the two earlier experiments. They show that the greatest effect on the urinary

nitrogen losses and the digestibility of the nitrogen occurs just after the low-nitrogen period. However, the effect of the low-nitrogen period undoubtedly influences the utilization of nitrogen by the lambs even after 20 days. In fact, even though the urinary nitrogen excretion may reach a fairly stable level within 30 days, the losses may still be lower for a considerably longer time; in other words, the lambs may be more efficient than if no low-nitrogen period of feeding had occurred.

Treatment of these data mathematically by means of the analysis of variance (18) showed no significant difference in the biological values obtained for the alfalfa ration and the one-third alfalfa and two-thirds timothy ration. Furthermore, this treatment of the data indicated no significant difference in nitrogen utilization between these two rations either before or after the low-nitrogen period. Similarly, there was no mathematically significant difference in the biological values for the soybean oil meal ration and the corn-gluten meal ration, either before or after the low-nitrogen period.

As previously mentioned, earlier experiments indicated a slightly lower efficiency of the nitrogen in the alfalfa ration as compared with the one-third alfalfa and two-thirds timothy ration. Since these additional data show no significant difference, it may be assumed that the earlier results were unduly influenced by one especially low value obtained for the alfalfa ration.

Also, earlier experiments involving three lambs had shown a slight superiority of the protein in the soybean oil meal ration as compared with the corn-gluten meal ration. Results obtained in this experiment indicate that the nitrogen of the corn-gluten meal ration was at least fully equal to that contained in the soybean oil meal ration.

#### GENERAL DISCUSSION OF RESULTS

The three experiments reported in this paper were planned to give specific answers to some of the questions which have arisen during the several years that quality of protein for ruminants has been under intensive study at this station. Although the results obtained apply specifically to the problems which arise when lambs are used, at least some of the same problems would arise with the use of other experimental animals.

The data reported in this paper indicate that a preliminary period of 10 to 12 days is sufficient for lambs to reach a more or less stable level of urinary nitrogen excretion after the feeding of a low-nitrogen ration. Apparently, this length of time is sufficient to take care of the lag in urinary nitrogen excretion which always follows when a marked change is made in the protein content of the ration. However, the authors of this paper are inclined to believe, along with certain other workers, that the urinary nitrogen losses determined during a collection period of sufficient length, even following such a preliminary period, may not necessarily represent the true endogenous losses. However, data have been obtained at this station on a fairly large number of lambs and the values tend to average consistently around a value of 0.037 gm. of endogenous nitrogen daily per kilogram of weight, even though there have been some marked variations within the data. To obtain these values, a low-nitrogen ration has been used, which furnished only the small amount



of protein that was present in the wheat straw which made up 25 percent of the total ration.

The low-nitrogen basal ration which has been used at this station is considered to be only partly satisfactory. It is not devoid of protein, and, at the present time, the authors have little hope of being able to formulate an entirely nitrogen-free ration for lambs that will be sufficiently palatable for use in such experimental work (8). Therefore, no direct check is possible to determine whether the small amount of straw protein affects the endogenous nitrogen values or whether the values obtained with this ration truly represent the endogenous level. Lambs fed the low-nitrogen ration containing 25 percent wheat straw are not capable of maintaining good appetites or their body weights. Feed consumption usually becomes irregular. Undoubtedly, some lambs respond so poorly to this ration that their nitrogen losses are not sufficiently reliable to be used safely.

The addition of dried skim-milk protein to the low-nitrogen basal ration in an amount to furnish 4 percent protein makes a more palatable ration. Because of the higher level of feed intake, as well as higher nitrogen intake, the lambs maintain their weights or make slight gains in weight. However, contrary to data obtained with rats (14), the addition of this protein does significantly affect the urinary nitrogen losses. The average value obtained from two lambs during the eleventh to twentieth day, inclusive, while fed the low-nitrogen basal ration was 0.0388 gm. per kilogram of body weight per day, while the value obtained from the same lambs and the same corresponding period on the nitrogen-poor ration containing approximately 4 percent dried skim milk protein averaged 0.0474 gm.

The addition of only enough dried skim milk or corn-gluten meal to furnish approximately 1.2 percent protein likewise improved the appetites of the lambs and their body weights. However, in no instance did these lambs have as strong appetites as those fed the 4 percent protein rations, nor could they maintain their body weights. The values for the endogenous nitrogen losses from lambs fed either dried skim milk or corn-gluten meal at the low levels of protein averaged 0.0438 gm. per kilogram of body weight.

There may be several reasons why lambs have responded differently than rats. Lambs are ruminants, and might be expected to respond differently to various proteins and amounts of protein entirely as a result of species difference. Moreover, egg rather than milk protein has been used largely in rations for rats. However, nitrogen balance experiments with lambs at this station (10) have shown that dried skim milk protein is as efficient as any common source of protein when compared at a 10-percent level. On the other hand, any particular protein that is of average quality at a 10-percent level of feeding might be utilized nearly 100 percent at a low level. If this is true, the quantity of protein furnished would affect the nitrogen metabolism of the animals more than would the quality. In the experiment reported in this paper, the protein of corn-gluten meal, fed at a 1.2-percent level, was apparently utilized with the same efficiency as the dried skim-milk protein similarly fed, although no conclusions were possible from the limited amount of data available. The data indicate that neither source of protein at either the 1.2-percent or the 4-percent level was completely utilized.

The metabolic nitrogen in the feces appeared to be affected but little by the addition of the small amounts of protein. The fecal nitrogen losses were higher on the average during the periods when skim-milk protein or corn-gluten-meal protein was included in the rations, but likewise the feed intake was greater. On the basis of dry-matter intake, the fecal nitrogen losses were similar to those found when the low-nitrogen basal ration was fed. Apparently, the digestibility of the protein was complete or nearly complete at these low levels.

Evidence was obtained in these experiments that subjecting lambs to a low-nitrogen ration influenced their utilization of nitrogen at a later date. Even though a 10-day preliminary period following the low-nitrogen period was apparently sufficient time to allow for the chief lag in nitrogen excretion following the change to a test ration, the effect of the preceding low-nitrogen ration still existed. These data indicate that the lambs were more efficient in their utilization of nitrogen because of a lower urinary nitrogen loss. On the other hand, there was a small though significant difference in the reverse direction in the digestibility of the protein.

The data indicate further that on the average lambs failed to reach a more or less stable level of urinary nitrogen losses until sometime after 20 days had elapsed. Furthermore, for at least 30 days after a low-nitrogen period, lambs were more efficient in the use of nitrogen than if they had not been subjected to a low-nitrogen period of feeding. For some time following a low-nitrogen ration, lambs apparently utilize nitrogen with an unusually high efficiency in order to regain nitrogen lost from the body, as well as to regain lost body weight. These experiments do not show definitely when this influence ceases to exist, although they indicate that the period of higher efficiency is probably influenced inversely by the length of time the lambs are fed the low-nitrogen ration or to an even greater extent by the feed intake of the lambs and their losses in body weight during this period. On the other hand, if lambs are in a severely low state of nutrition at the close of the low-nitrogen period, they may not respond well to a ration containing ample protein.

The relative value of the nitrogen in one ration as compared with the value of the nitrogen in another is apparently not influenced by a low-nitrogen period of feeding. Therefore, even though the values obtained for the nitrogen utilization following a low-nitrogen period would tend to be higher than could be expected normally, the values would have a comparative significance with others obtained in the same manner, provided the values were obtained after the same length of time had elapsed since the low-nitrogen period.

On the basis of these experiments, the authors believe certain modifications should be made in the former methods used in determining biological values of proteins for lambs. If direct values for endogenous and metabolic nitrogen are to be determined for each lamb, the necessary period of low-nitrogen feeding should be conducted at the end of the experiment and not preceding or during the course of the experiment. Following this period on a low-nitrogen ration, the lambs should be discarded from future experimental use.

Also, the authors believe that average values for the endogenous and metabolic nitrogen, as determined from a sizeable number of

lambs of comparable age and weight, may be fully as desirable to use in calculating biological values as individual values obtained directly for each experimental lamb. In the grouping of data, abnormally low or high values that may be explained on the basis of extremely low feed intake, or other known factors, could be discarded. On the other hand, it is difficult to discard data for an individual lamb in a small experiment, even though the values appear questionable. The variation in values for endogenous and metabolic nitrogen losses which commonly appear are probably due more to the manner in which the lamb responds to the ration, as shown by differences in feed intake, health, and condition of the animal, or loss in body weight, than to the possible intrinsic differences between lambs in their endogenous losses.

The authors prefer to emphasize the percentage of total nitrogen retained and the percentage of digested nitrogen retained rather than biological values in expressing nitrogen utilization by lambs. It is appreciated that biological values accurately determined would give a more quantitative measurement of the utilization of the digested nitrogen. However, the other methods of expressing the efficiency of protein furnish information of as much or more practical value and can be determined for lambs with less experimental error. Fortunately, in the series of experiments conducted at this station the comparative values between rations have been the same whether expressed in terms of the retention of total nitrogen, the retention of digested nitrogen, or biological values. In this connection, it should be pointed out that no matter whether the results are expressed in terms of biological values or of percentages of total or digestible nitrogen retained, it is essential that there be the same percentages of protein in all rations that are compared.

The experiments reported in this paper have furnished information upon the variation within short periods of the urinary and fecal nitrogen excretions of lambs. On inspection of these data, it seems probable that collection periods of considerable length are desirable. There may be a doubt as to whether or not 10-day collection periods are long enough in nitrogen balance studies with lambs.

#### SUMMARY

Three nitrogen balance experiments have been conducted with growing wether lambs to study certain problems connected with the determination of biological values of protein. Specifically, the major problems studied were: (1) The length of time required by lambs to reach an endogenous nitrogen level when fed a low-nitrogen ration, (2) the effect on feed consumption, on loss in body weight, and on nitrogen excretion of adding a small amount of dried skim milk or corn-gluten meal to the low-nitrogen basal ration, (3) the time required by lambs to reach a stable level of nitrogen utilization when fed rations containing 10 percent or more protein following the feeding of a low-nitrogen ration, and (4) the influence of a low-nitrogen ration on the accuracy of biological value as heretofore obtained with lambs.

The data reported in this paper indicate that a preliminary period of 10 to 12 days was sufficient for lambs to reach a more or less stable level of both urinary nitrogen and fecal nitrogen excretion following

the feeding of a low-nitrogen ration. Although the minimum urinary nitrogen losses may not have been entirely reached within this time, the chief reduction in urinary nitrogen losses had occurred.

The addition of dried skim-milk protein to the low-nitrogen basal ration in sufficient amounts to furnish approximately 4 percent or 1.2 percent protein increased the palatability of the rations and resulted in less loss of weight. However, the urinary nitrogen losses were greater when the dried skim milk was fed at either level than when the basal ration was fed. Apparently, the nitrogen of the dried skim milk was not completely utilized even at these low levels. The results in regard to fecal nitrogen losses indicate that the dried skim-milk nitrogen at each level was completely digested and did not affect the metabolic nitrogen losses.

These experiments show further that feeding lambs a low-nitrogen ration influences the utilization and digestibility of nitrogen at a later date. Lambs were more efficient in their utilization of nitrogen because of a lower urinary nitrogen loss for at least 30 days after the low-nitrogen period of feeding of 20 days. There was a small though significant difference in digestibility, with the protein being less digestible for the lambs following the low-nitrogen period of feeding. However, the relative efficiency of the nitrogen between rations was not influenced when all comparisons were comparable in regard to time following the low-nitrogen period of feeding. The biological values as determined for four rations following a low-nitrogen period of feeding were higher than the biological values determined for these same rations and by the same lambs prior to a low-nitrogen period.

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# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Respiration and Oxidase and Catalase Activity of Apples in Relation to Maturity and Storage (Key No. G-1267) - - - - -	453
BOYCE D. EZELL AND FISK GERHARDT	
Factors Determining the Reduction in Yield of Field Corn by the European Corn Borer (Key No. K-318) - - - - -	473
L. H. PATCH, G. W. STILL, M. SCHLOSBERG, AND G. T. BOTTGER	
Hydrocephalus, a Lethal in Cattle (Key No. Mich.-38) - - - - -	483
C. L. COLE AND L. A. MOORE	
Development of Synthetic Food Media for Use in Nutrition Studies of the European Corn Borer (Key No. K-319) - - - - -	493
G. T. BOTTGER	



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# JOURNAL OF AGRICULTURAL RESEARCH

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## RESPIRATION AND OXIDASE AND CATALASE ACTIVITY OF APPLES IN RELATION TO MATURITY AND STORAGE<sup>1</sup>

By BOYCE D. EZELL, *associate physiologist*, and FISK GERHARDT, *physiologist*,  
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### INTRODUCTION

Certain physiological changes that are taking place in fruit at the time of harvest continue after it is placed in cold storage. The immediate effect of low temperature is to retard these processes. If all were retarded equally, it might be supposed that the fruit would continue to ripen normally, but at a much slower rate. If, however, the different processes should be unequally retarded, then various disorders might result. That many of the physiological diseases of fruits in storage may be due to an unbalanced condition brought about by an unequal retardation or stoppage of the various life processes by low temperature appears probable. The failure of Bosc or Comice pears to ripen normally after prolonged storage seems almost certainly to be caused in this manner. The enzymes in the pears that change protopectin to soluble pectin no longer function adequately and the fruit remains hard and unpalatable even when external conditions are favorable for ripening. That all the life functions have not ceased is readily shown by the continued production of carbon dioxide. Soft scald, scald, and break-down of apples, and core break-down of pears are other cold-storage disorders that may have their origin in an unequal slowing down of the various life processes.

If all these processes are not retarded equally, one might suppose that the more rapidly they are taking place at the time of storage the greater would be their divergence from normal and the more serious the results. There is some evidence to support this supposition. If Jonathan apples are delayed at high temperature between harvest and cold storage, the life processes, as measured by carbon dioxide production, are speeded up, and the fruit is also more subject to soft scald when stored at 32° F. A more striking example, probably caused by unequal retardation of different processes may be observed with Bartlett pears. If pears of this variety are delayed at warm temperatures for a few days until the ripening processes are well under way and are then stored at 40°, large amounts of acetaldehyde are produced in the tissues, accompanied by a disagreeable odor, excessive scald, and core break-down.

In previous work (4, 5)<sup>2</sup> in which various physiological factors connected with the development and maturity of Bartlett pears were studied, it was shown that the oxidase activity of the fruit decreased throughout the growing season. The curves for catalase and respiratory activity were U-shaped. The low point on these curves occurred at or near the time the fruit should be harvested for best storage and

<sup>1</sup> Received for publication February 23, 1942.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 470.

dessert quality. These results might indicate that the rate of metabolic activity at the time of harvest and storage influenced the subsequent behavior in storage.

The present work was undertaken to determine whether there is a period at which the various life processes in apples are at a minimum and during which the fruit could be harvested and stored with less danger from the disorders that often develop in storage. The indexes of metabolic activity used were respiration and oxidase and catalase activity. These studies, extending over two seasons, were made at Wenatchee, Wash. For a review of the literature on respiration and enzyme activity, the reader is referred to the publications previously cited (4, 5).

#### MATERIALS AND METHODS

In order that the fruit throughout the season might be uniform and representative of the changes normally occurring as the fruit matures, representative trees in good vigor were selected, and the fruit was taken from these trees throughout the season. The maturity range included fruit sampled from early July to November. Because of factors over which the writers had no control, it became necessary during the season of 1938 to change the orchard from which the Delicious and Golden Delicious varieties were taken. Fruit of these two varieties harvested on September 6 and thereafter was taken from a different orchard, located in a slightly later maturing district, from those harvested previous to that time. The other varieties were taken from the same trees throughout the season, and the Jonathans from the same trees throughout both seasons.

The methods used in determining the rate of respiration and the catalase activity were the same as those used previously (5). In 1937 the oxidase activity was determined iodometrically by the method described by Guthrie (9) and in 1938 by that described later by Ezell and Gerhardt (6). The rate of growth or weight increase was determined by the average weight of representative fruits taken for the carbon dioxide determinations at each period of sampling. The rate of respiration is reported as milligrams of carbon dioxide per kilogram per hour, catalase as cubic centimeters of oxygen liberated in 5 minutes by 5 cc. of juice, and oxidase as cubic centimeters of N/100 sodium thiosulfate per 2 cc. of juice.

#### EXPERIMENTAL DATA

##### RATE OF RESPIRATION AFTER HARVEST

Early in the work it became evident that the rate of respiration of the fruit changed rather rapidly after harvest. The rate and direction of this change were not uniform throughout the season. Some delay after harvest is inevitable. A representative sample must be selected from the trees, the fruit must be brought to the laboratory and placed in the respiration chamber, and the chamber must be sealed and cleared of the carbon dioxide normally present in the air. Moreover the temperature of the fruit at harvest will vary with the air temperature, and it should be brought to room temperature before carbon dioxide determinations begin. Throughout both seasons the fruit was harvested between 8:30 and 9:00 a. m. and all respiration determinations were made at 67° F. unless otherwise specified. Where

cold storage is specified, a temperature of 32° was used. In table 1 the rate of respiration of Jonathan apples at 67°, shown for various periods after harvest, changes rather rapidly. The amount of change during the first 5 hours is unknown but doubtless is appreciable. No determinations were attempted during this period, since 5 hours after harvest was believed to be the minimum time necessary under the conditions of these experiments for setting up the respiration chambers, clearing the container of the carbon dioxide normally present in the air, and bringing the fruit to the temperature of the storage room. When the differences between the temperatures of the fruit and the storage room were large, as on October 18 and 20 and November 7, even more time might have been desirable.

TABLE 1.—Rate of respiration of Jonathan apples at 67° F. during various periods after harvest

Date	Period after harvest	Respiration as CO <sub>2</sub> per kilogram per hour	Date	Period after harvest	Respiration as CO <sub>2</sub> per kilogram per hour
	<i>Hours</i>	<i>Milligrams</i>		<i>Hours</i>	<i>Milligrams</i>
Sept. 8..	6 and 7.....	19.12	Sept. 9..	32 to 34, inclusive.....	13.14
8..	12 to 14, inclusive.....	17.20	10..	49 to 51, inclusive.....	15.47
9..	25 to 27, inclusive.....	15.55	11..	74 to 76, inclusive.....	16.87

Figure 1 shows graphically the rate of respiration after harvest throughout the growing season of the four varieties of apples studied during 1938. In arriving at the values plotted, the carbon dioxide determinations began at the end of 5 and 24 hours and usually ran for 3 hours. The values plotted are the average for the 3 hours. These values show that the direction and the amount of changes were not uniform throughout the season and that both may vary with the variety. However, in general it may be said that during the early part of the growing season the rates of respiration of the varieties studied decreased in the period between the 5-hour and 24-hour determinations. During the middle part of the season an increase took place during this time in all the varieties except Jonathan, and in the latter part of the season an increase occurred in all varieties during this period.

These results are of interest because they show that the time elapsing between harvest and the first determination of respiration may alter considerably the shape of the early part of the respiratory curve, and unless the elapsed time is known and kept constant, erroneous conclusions may be drawn when comparing different maturities or varieties. Also, if the complete picture of the respiratory curve of fruit in storage is to be obtained, it is necessary that determinations be made at shorter periods after harvest than has sometimes been done in the past.

The air temperature before harvest might be expected to influence the rate of respiration to some extent. If so, the effect seems to have been largely lost, or relegated to a position of minor importance, within 5 hours. The minimum temperature the night before harvest was approximately 45° F. on August 8, September 8, and October 6; yet on August 8 and September 8 the respiration of Jonathan apples decreased between the 5-hour and the 24-hour determination and

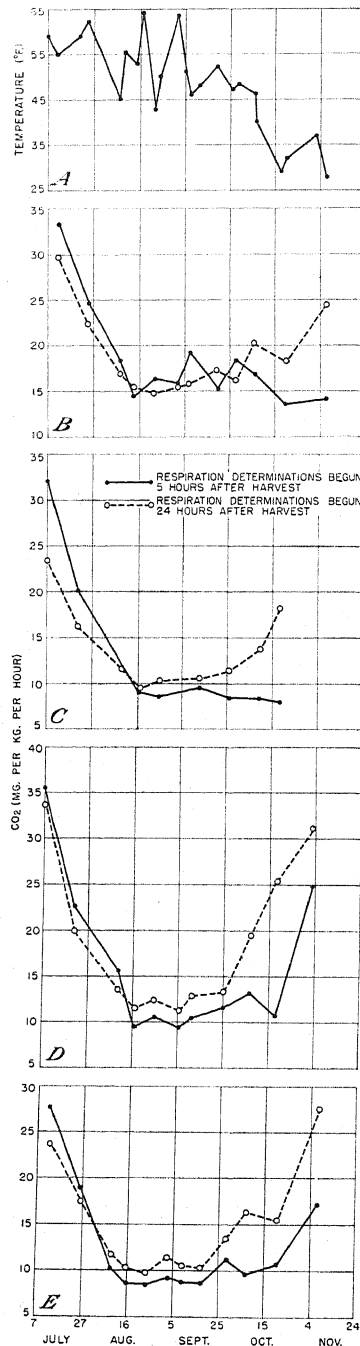


FIGURE 1.—Minimum temperature on night before harvest (A) and changes in the rate of respiration of Jonathan (B), Delicious (C), Golden Delicious (D), and Yellow Newtown (E) apples during the 1938 growing season.

increased on October 6. Respiration of Yellow Newtown increased during this time on all dates, but the amount of increase was much greater on the latter date. Tests showed that the internal temperature of the fruit had reached the temperature of the storage within 5 hours under the conditions of these experiments and thus precludes the possibility that the difference in respiration was due to a difference in the temperature of the fruit.

In order to determine the amount of variation to be expected in sampling and in the measurement of the respired carbon dioxide, triplicate lots of Yellow Newtown apples were harvested on August 8 and the rate of respiration was determined. These results (table 2) may be taken as an indication of how closely the composite samples used in the respiration experiments reflect the rate of respiration of all the fruit on the tree. Their uniformity precludes the possibility of the wide differences in respiration during the season being due to errors in sampling and in the measurement of the respired carbon dioxide.

#### RATE OF RESPIRATION AND ENZYME ACTIVITY DURING THE GROWING SEASON

During 1937 Jonathan and Winesap apples grown at elevations of 850 and 2,400 feet were used. Samples were taken at intervals from July 14 to November. On November 2 approximately 90 percent of the Winesaps had dropped, and sampling of this variety for respiratory activity was discontinued. The last sampling of Jonathans for respiratory activity was taken November 15, approximately 8 weeks after commercial harvest. One additional sampling for enzyme activity was made where fruit was available. Figures 2 and 3 show the rate of respiration and oxidase and catalase activity of the two varieties.



TABLE 2.—Rate of respiration in triplicate 36-fruit lots of Yellow Newtown apples

Date	Period after harvest	Respiration as CO <sub>2</sub> per kilogram per hour		
		Lot A (2,792 gm.)	Lot B (2,568 gm.)	Lot C (2,758 gm.)
	<i>Hours</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>
Aug. 8	7 to 9, inclusive	10.08	10.11	10.61
9	24 to 26, inclusive	11.57	11.91	11.65
9	27 to 29, inclusive	11.65	12.00	11.48

With Jonathans the rate of respiration decreased in the early part of the season and reached a minimum about the middle of September at approximately the time the fruit would be harvested commercially. The rate of respiration then increased as maturity advanced and finally decreased late in the season. The final decrease started when vascular water core became evident in appreciable amounts; slight water core was noticeable 10 days earlier at the higher elevation. Oxidase activity decreased until past the commercial harvest season and then increased slowly. This increase coincided with the development of water core in the tissue and may have been caused by the accumulation of anaerobic respiratory products in the waterlogged tissues; alcohol has been shown to exert a stimulatory action on oxidase activity (5). Catalase activity increased throughout the season except at the last sampling at the low elevation, at which time the fruit was showing medium to severe tissue and vascular water core.

The Winesap variety behaved similarly to Jonathan. The respiration decreased at first and later increased; oxidase activity decreased and then increased as maturity advanced. The Winesap watercored severely and dropped from the tree, so that it was impossible to continue the test much past the commercial harvest season.

Since fruit grown at 850 feet and 2,400 feet showed the same general trend in both varieties, only fruit grown at 850 feet was used in 1938, and four varieties (Jonathan, Delicious, Golden Delicious, and Yellow Newtown) were studied. Jonathan and Golden Delicious are susceptible to soft scald; Delicious and Yellow Newtown are not. However, Delicious is susceptible to storage scald, especially if harvested before it is fully mature. Beginning early in July, respiration and oxidase and catalase activity of the four varieties were followed during the remainder of the season. The results are plotted in figures 4 to 7.

It will be seen from figures 4 to 7 that at the beginning of the test the rate of respiration was comparatively high in all varieties, but that it decreased rapidly, approaching a minimum around the middle or latter part of August, several weeks before the fruit was ready to be harvested commercially. Respiration remained near the minimum for several weeks, and in the case of Jonathan and Delicious for the rest of the season, if the rate beginning 5 hours after harvest is taken as the accepted one. In Yellow Newtown and Golden Delicious, however, respiration increased in the latter part of the season. The respiration as determined 24 hours after harvest increased in all varieties late in the season, and the curves based on the data are U-shaped.



FIGURE 2.—Rate of respiration beginning 5 hours after harvest (A) and catalase activity (B) and oxidase activity (C) during the growing season of Jonathan apples grown at two elevations, 1937.

Oxidase activity decreased from early July until the fruit was ready for harvest. In Jonathan and Yellow Newtown oxidase activity continued at this low rate, but in Delicious and Golden Delicious oxidase activity increased near the end of the sampling period, after the commercial harvest season was over.

Contrary to its behavior in Bartlett pears (4), catalase activity started low in all varieties and increased as the season progressed. In Jonathan and Yellow Newtown the increase was slow and uniform throughout the season, but in Delicious and Golden Delicious the rate of increase was more rapid in the latter part of the season. This period of rapid increase started before the fruit was commercially mature and continued as long as the fruit remained on the tree. The magnitude of catalase activity late in the season was also much greater in Delicious and Golden Delicious than in Jonathan and Yellow Newtown.

#### SEASONAL DIFFERENCES IN RESPIRATION AND ENZYME ACTIVITY

Jonathan apples from the same trees were used during two seasons. The rates of respiration and the oxidase and catalase activity are shown in figure 8. It will be seen that although there are variations, the general trends are similar for the 2 years. Catalase started low and increased throughout the season. At the time of

the first sampling the activity of this enzyme was about the same in the 2 years, but in 1937 the increase was more rapid and reached a greater height. The oxidase activity decreased during both seasons, and, although the curves indicate a close correlation between the two seasons, in reality the activity in 1938 was relatively less, as the ascorbic acid substrate used that year is more readily oxidized than the glucose-derivative substrate used the previous year (6). The increase that occurred late in the 1937 season was not so evident in 1938. Respiration started at a high rate and decreased rapidly until mid-August, and although there was some variation between sampling dates, in general the respiratory activity remained near this level in 1938. In 1937 respiratory activity dropped in late September and then increased more than doubling the minimum rate within 30 days. After this rise it decreased about as fast as it had increased. This increase coincided with, or followed closely, the appearance of water core, which became evident in early October and was medium to severe by October 22. The following year the water-cored condition was not evident quite so early, but it had become severe by October 20, without resulting in an

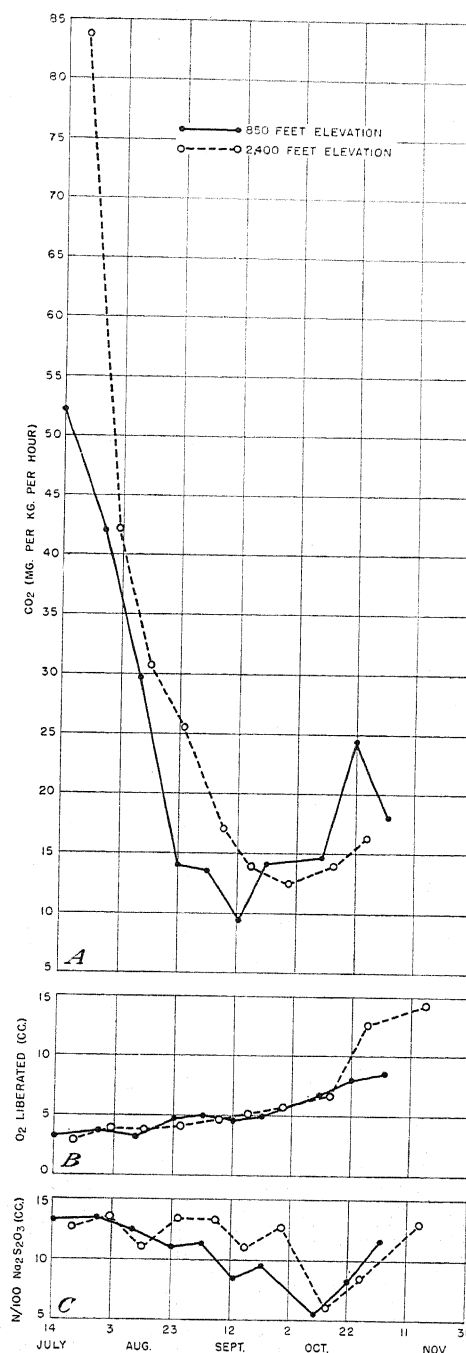


FIGURE 3.—Rate of respiration beginning 5 hours after harvest (A) and catalase activity (B) and oxidase activity (C) during the growing season of Winesap apples grown at two elevations, 1937.

increased rate of respiration. Thus the effect, if any, of the water core on respiration is not clear.

#### EFFECT OF MATURITY AT HARVEST ON RESPIRATORY AND ENZYME ACTIVITY IN STORAGE

To determine the effect of maturity on the respiratory and enzyme activity at harvest and on their subsequent behavior in storage, Jonathan, Delicious, Golden Delicious, and Yellow Newtown apples

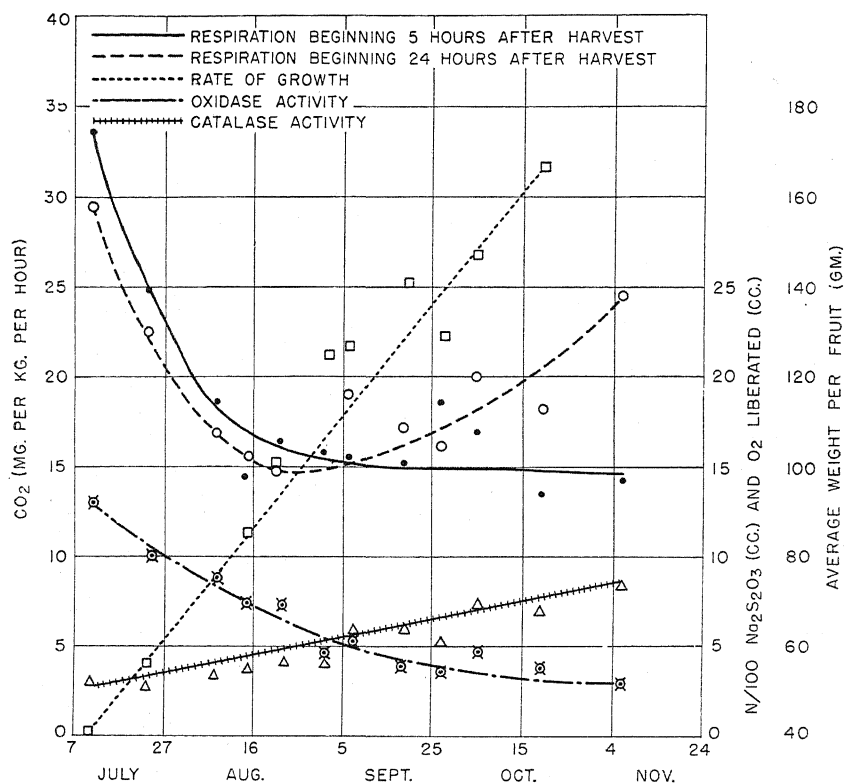


FIGURE 4.—Respiration rate, oxidase and catalase activity, and rate of growth of Jonathan apples during the growing season, 1938.

were picked at the beginning of, toward the middle of, and at or near the end of the commercial harvest season and placed immediately in storage at 32° F. The dates of harvesting and pressure tests are given in table 3. Figures 9 to 12 show the rate of respiration and enzyme activity in storage of fruit of the three maturities. Fruit picked for storage at the beginning and near the middle of the commercial harvest season showed little difference in rate of respiration in storage, although that of the second picking showed a slightly higher initial rate (determined 1 day after storage at 32°) and in general was slightly higher in storage. The third picking, made after most of the fruit had been picked commercially, showed definitely



greater initial activity and also a greater activity in storage than the two earlier pickings. Fruits of this maturity also showed the greatest range in respiration in storage. The greatest catalase activity and the lowest oxidase activity in storage were usually found in the fully matured fruit.

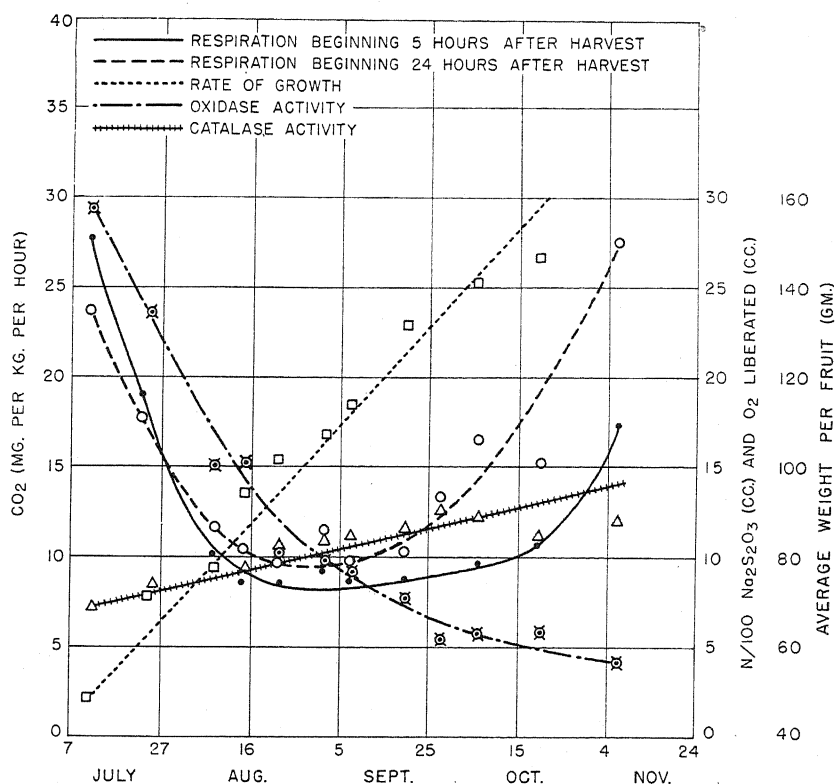


FIGURE 5.—Respiration rate, oxidase and catalase activity, and rate of growth of Yellow Newtown apples during the growing season, 1938.

TABLE 3.—Date of picking and pressure test of fruit stored at 32° F. immediately after picking

Variety	Picking		Pressure test	Variety	Picking		Pressure test
	Order	Date			Order	Date	
			Pounds				Pounds
Delicious	First	Sept. 12	17.1	Jonathan	First	Sept. 8	17.6
	Second	Sept. 26	16.1		Second	Sept. 20	16.3
	Third	Oct. 18	14.8		Third	Oct. 20	15.7
Golden Delicious	First	Sept. 12	17.4	Yellow Newtown	First	Sept. 16	20.5
	Second	Sept. 26	16.0		Second	Oct. 6	18.0
	Third	Oct. 18	14.0		Third	Oct. 20	17.8

## DISCUSSION

Results from this study indicate that there is no sharply defined period at which the metabolic activity of apples, as indicated by respiration and oxidase and catalase activity, is at a minimum and at

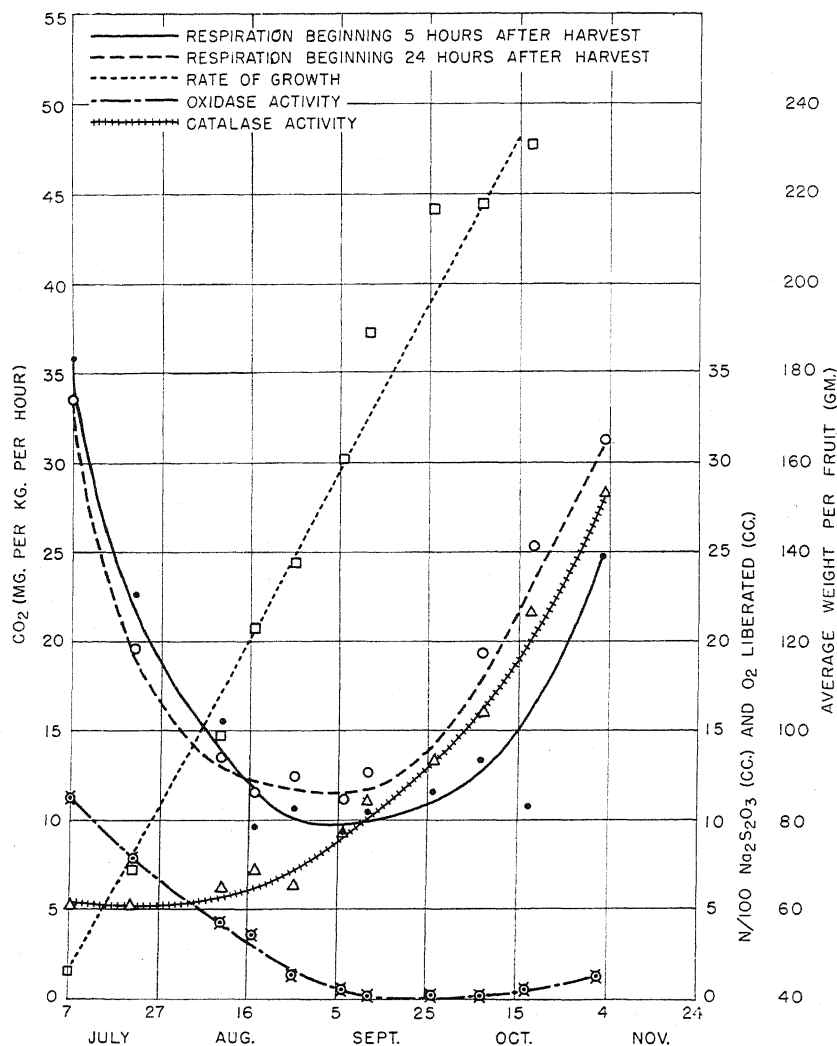


FIGURE 6.—Respiration rate, oxidase and catalase activity, and rate of growth of Golden Delicious apples during the growing season, 1938.

which the fruit may be harvested and stored with least danger from physiological disorders. As the fruit matures the values for the rate of respiration, as determined 24 hours after harvest, form a U-shaped curve, but the base of the curve is rather broad and is reached before the fruit is ready for harvest.

In the present study it was shown that the time elapsing between harvest and the measurement of the respired carbon dioxide affects the rate of respiration and may change the shape of the respiration

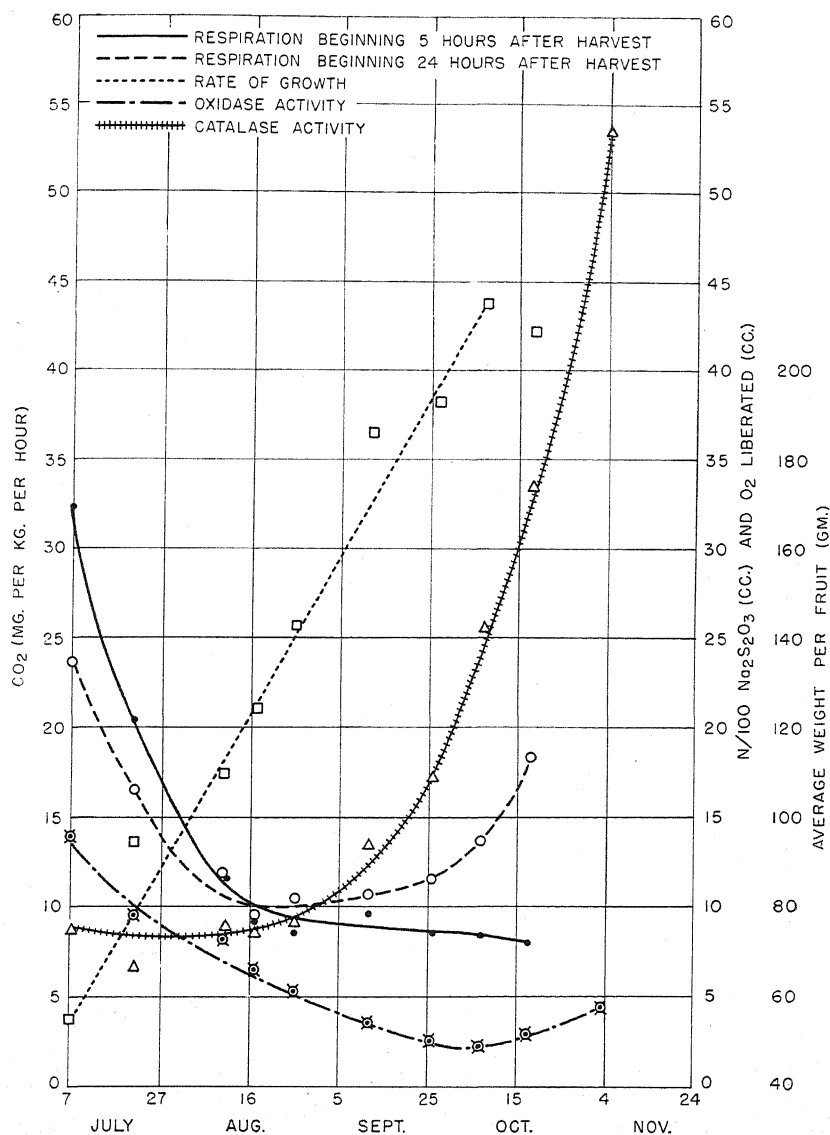


FIGURE 7.—Respiration rate, oxidase and catalase activity, and rate of growth of Delicious apples during the growing season, 1938.

curve. If the measurement is begun at the end of 5 hours after harvest, Delicious does not show the increase in the latter part of the growing season, and consequently the curve for the values is not U-shaped (fig. 7). In Golden Delicious (fig. 6), in Yellow Newtown

(fig. 5), and in Winesap (fig. 3) a definite increase is noted even after 5 hours. With Jonathan an increase is noted in one season (fig. 2) and none in the other (fig. 4). But if the period elapsing is extended through 24 hours, definite U-shaped curves are observed in all cases.

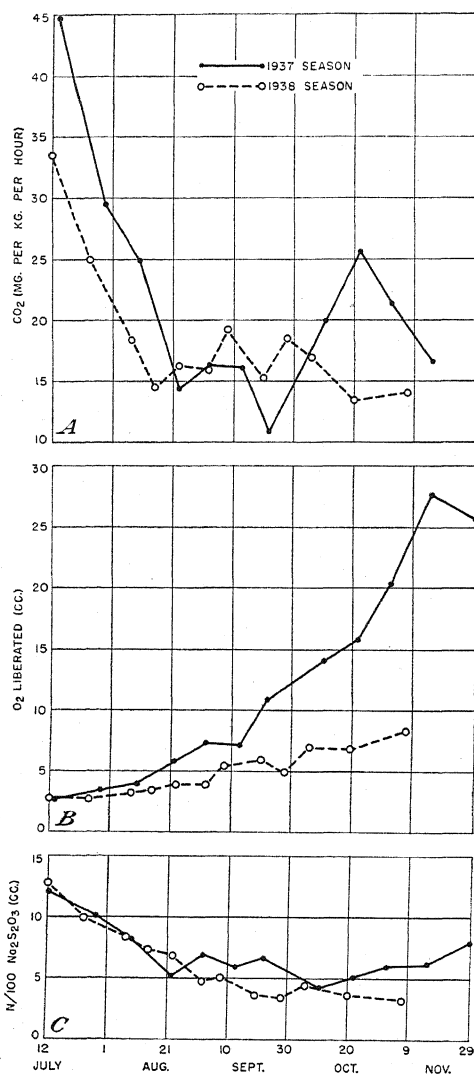


FIGURE 8.—Seasonal differences in rate of respiration (A), in catalase activity (B), and in oxidase activity (C) of Jonathan apples.

senescence. Gustafson (8) found that with tomatoes the carbon dioxide production reached a minimum about the time of cessation of growth and then increased to a maximum when the fruits were orange to red in color. Finally there was a subsequent decrease "as the tomato approaches a condition of a mixture of carbohydrate and

Even where an increase is observed after 5 hours, the increase is much greater after 24 hours. Gore (7) reported that there was no stimulus in rate of respiration of peaches due to picking from the tree. If this is also true for apples, then it seems logical to assume that the sooner after harvest the measurement is made the more closely it should represent the rate at harvest, and it is possible that if the determination were made immediately after harvest no increase during the season would be noted in any variety. However, a U-shaped curve, determined 5 hours after harvest, has previously been reported for Bartlett pears (5).

Other workers also have reported a decrease in respiration during the growth of the fruit with a later increase as the fruit approached maturity. Kidd (1) stated that respiratory activity of apples is greatest at the beginning of fruit formation, falls to about one-fifth at the end of cell division (fruit about the size of a walnut), and continues to decrease slowly during cell enlargement. During maturation a critical change in respiratory activity occurs, and there is a sharp rise followed by a slow decline during



water." In his work approximately one-half hour intervened between harvest and the beginning of the respiration determinations. Matsumoto (15) reported that with peaches the minimum rate of carbon dioxide production occurred at the period of maximum growth

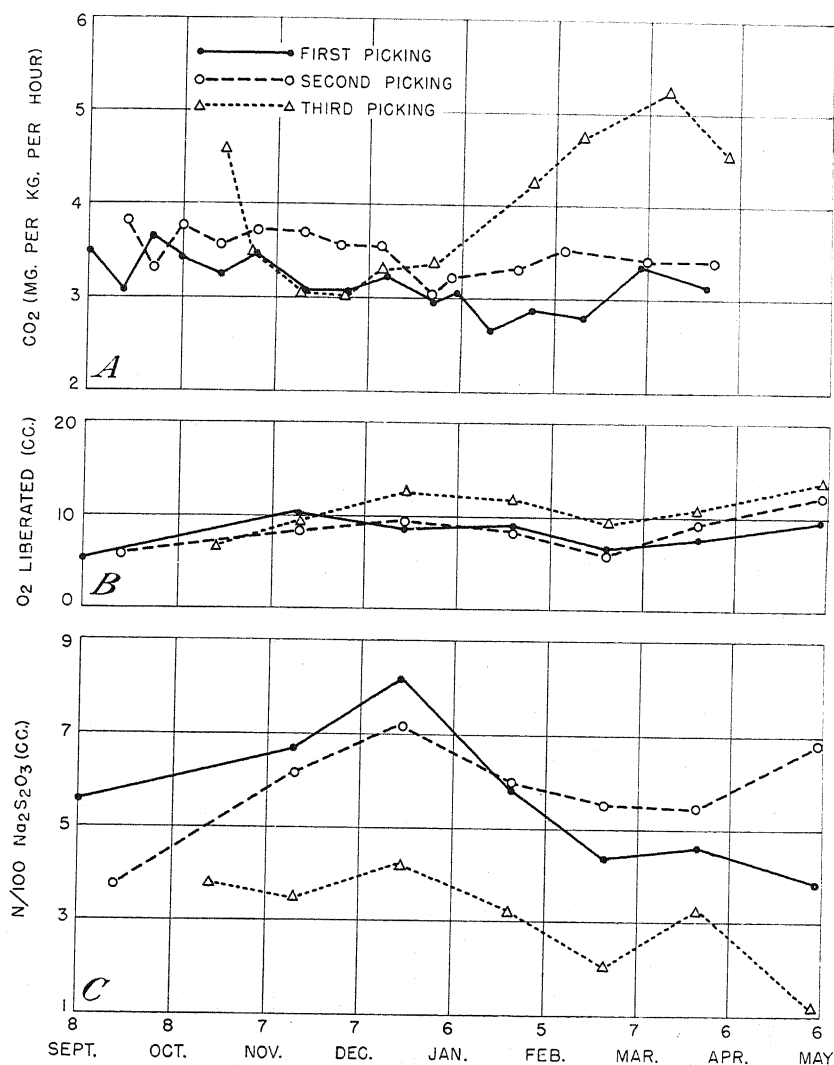


FIGURE 9.—Effect of maturity at harvest on rate of respiration (A), on catalase activity (B), and on oxidase activity (C) of Jonathan apples during cold storage.

and then increased until the fruit was overripe, 15 or more days later. Unfortunately, he did not state how soon the respiration studies were started after the fruit was picked. He also reported that pears and apples showed a similar trend in respiration, but that with Satsuma oranges the rate of respiration was lowest in the least mature fruit and

gradually increased as the fruit approached maturity. However, since his data for Satsumas covered only the last 3½ months of the growing season of the fruit, it appears quite probable that had he extended his observations over a greater part of the growing period

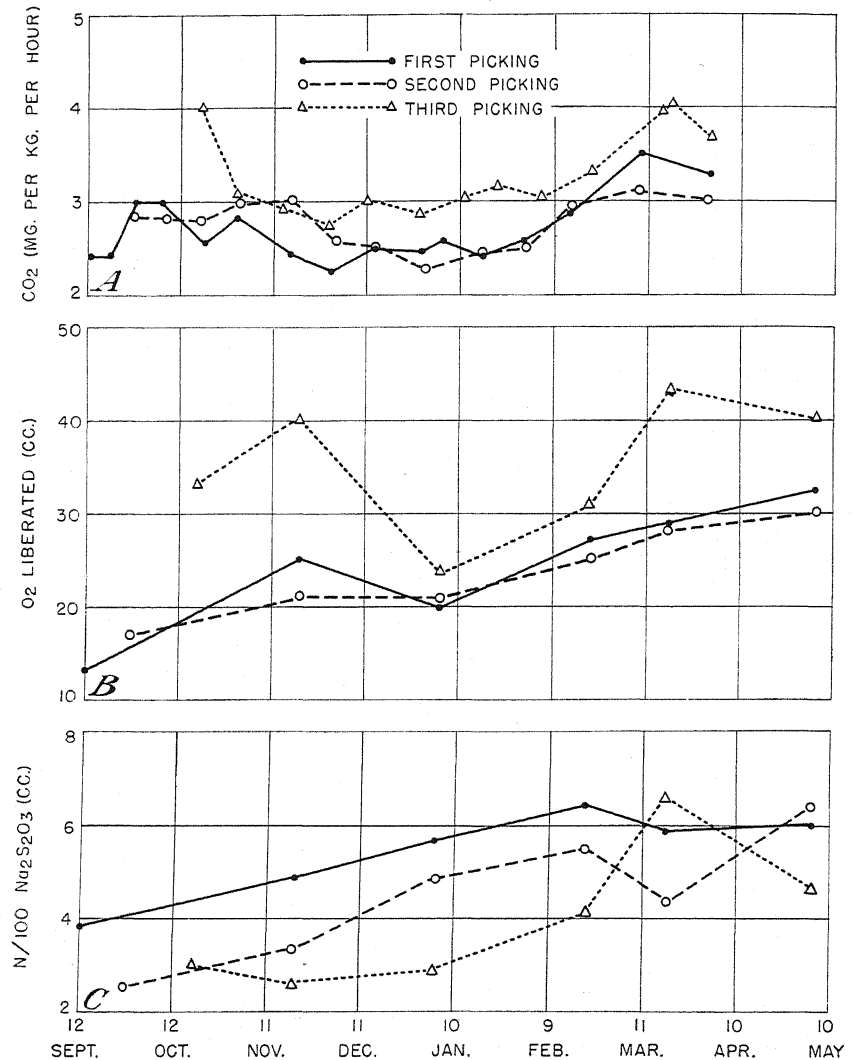


FIGURE 10.—Effect of maturity at harvest on rate of respiration (A), on catalase activity (B), and on oxidase activity (C) of Delicious apples during cold storage.

the curve obtained would also have been U-shaped. That this is true is suggested by the fact that the growth-rate curve of Satsuma oranges declines throughout the period covered. His data with the other fruits show that the growth rate begins to decline about the time the respiration begins to increase.

This change in rate of respiration of fruit during the harvest season and the subsequent effect in storage assume added interest when viewed from the storage standpoint. The rate of respiration determines the amount of heat generated by the fruit and must be taken into consideration in determining the amount of refrigeration necessary for properly storing or precooling the product. The results reported in this study show that the rate of respiration, and consequently the heat of respi-

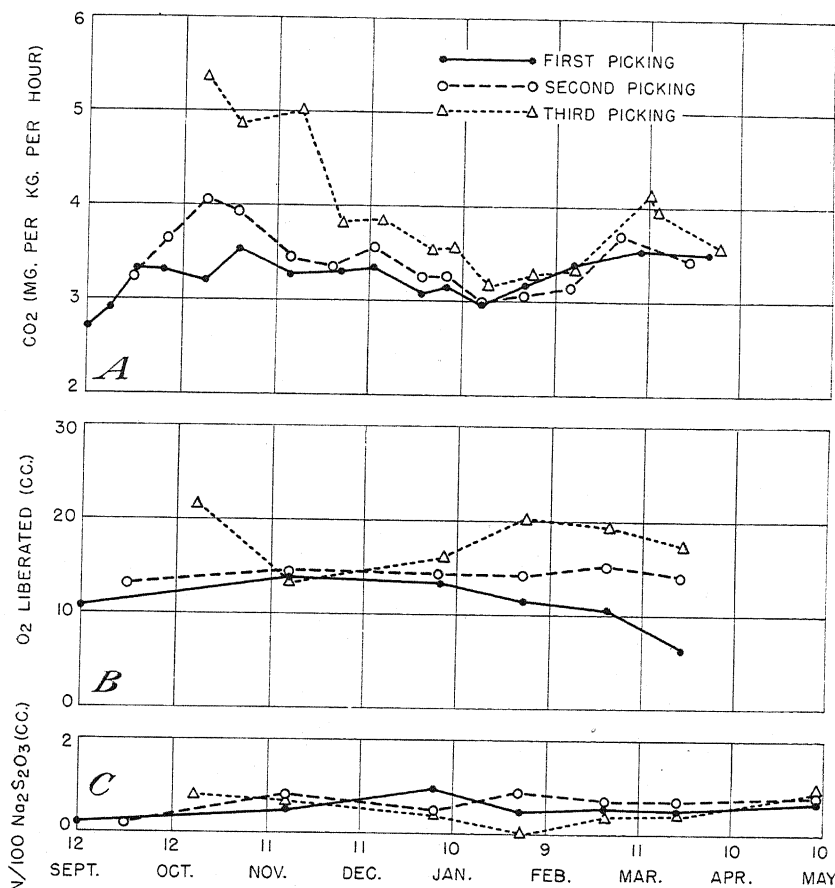


FIGURE 11.—Effect of maturity at harvest on rate of respiration (A), on catalase activity (B), and on oxidase activity (C) of Golden Delicious apples during cold storage.

ration, is not a stationary and definite figure for apples. Maturity, as well as the time elapsing between harvest and storage, exerts a considerable influence, and more refrigeration will be necessary for fully mature fruit than for fruit picked at a less mature stage.

In the present work the respiration measurements were made at a higher temperature than the minimum air temperature the night before harvest (fig. 1). Several workers have noted that when plant

tissues are moved from a lower to a higher temperature the rate of respiration increases to a point above that at which the tissues would normally respire at the higher temperature but that it later falls to the rate normal for the temperature. Willaman and Brown (16),

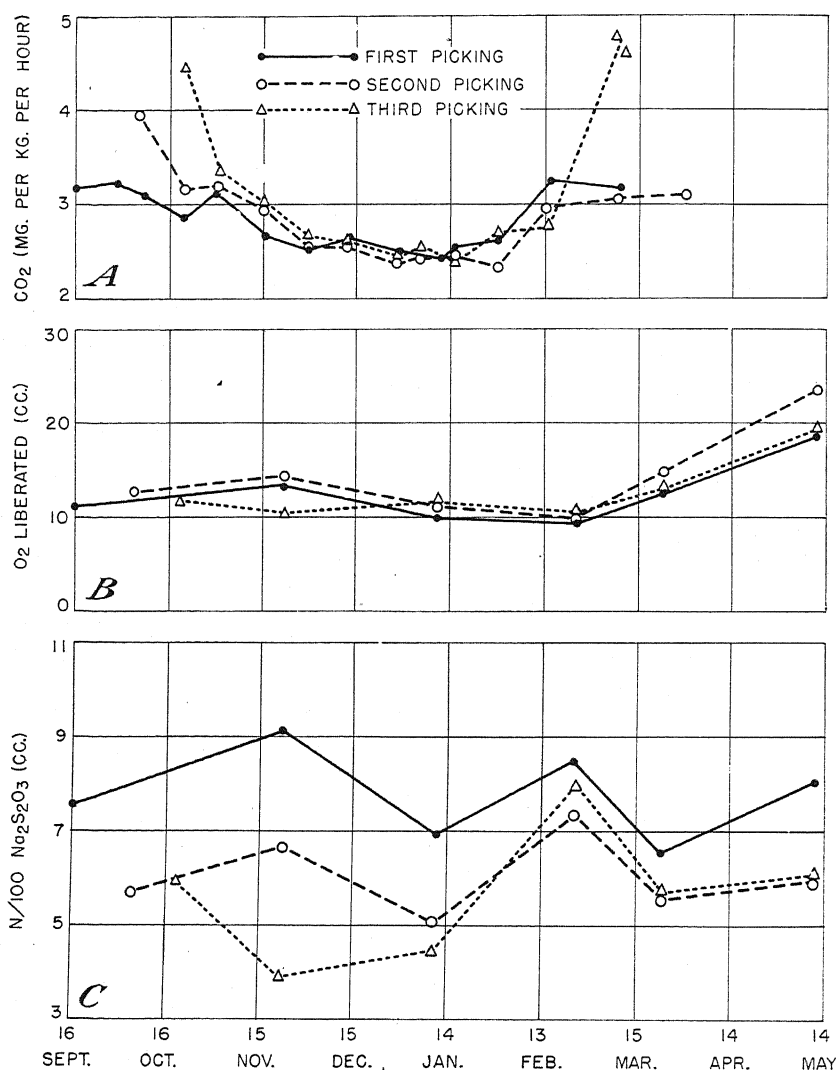


FIGURE 12.—Effect of maturity at harvest on rate of respiration (A), on catalase activity (B), and on oxidase activity (C) of Yellow Newtown apples during cold storage.

working with apple twigs, ascribed their results to a lower solubility of the carbon dioxide in the cell sap at the higher temperature, with a consequent release of the excess gas. Magness and Ballard (13) found no increase with Bartlett pears other than could be accounted for by the slightly riper condition of the cold-storage fruit. Harding



(10) studied the effect of alternating temperatures on the rate of respiration of Grimes Golden apples and concluded that "The rate \* \* \* was neither stimulated nor depressed further (after the fruit samples had reached temperature equilibriums of 50° and 30° F.) than the respiration rate of fruit held constantly at the two temperatures." Magness and Burroughs (14) noted no difference with Wine-sap and Baldwin apples that could be ascribed to stimulation by low temperatures. However, Burroughs (3) reported some stimulatory effect on Wagener apples, especially in immaturesly picked fruit. Kimbrough (12) reported a definite increase in respiration with potatoes, more excess carbon dioxide being given off than could be dissolved in the water of the potato at the storage temperature. He also determined the ratio of carbon dioxide to oxygen of the increased respiration and found it to be approximately 1, indicating that the excess was due to increased respiration rather than to dissolved carbon dioxide. Appleman and Smith (2) studied the effect of previous cold storage on a number of vegetables and found a definite stimulation with potatoes but no detectable increase with carrots. They concluded that the degree of stimulation varied with the vegetable and that those vegetables in which the percentage of starch was relatively high and in which there was a rapid shifting of the carbohydrate equilibrium with temperature changes were the ones that showed the greatest increase in initial respiration when they were transferred from a lower to a higher temperature. With potatoes Kimbrough (12) found that a storage period of about 3 weeks at the low temperature was necessary for the maximum stimulation when the tubers were moved to a higher temperature, and that the maximum respiration occurred on the second or third day after removal.

In the discussion of figure 1 (p. 456) it was pointed out that the minimum temperature the night before harvest was approximately 45° F. on August 8, September 8, and October 6, but that the respiration of Jonathan apples decreased in the period between the 5-hour and the 24-hour determination in the first two instances and increased in the last. This difference might indicate that maturity affects the time necessary for stimulation to take place. This conclusion, however, is opposed by the fact that the respiration of Yellow Newtown, a later maturing variety, increased in each instance. The divergence between the 5-hour and the 24-hour periods was greatest in the latter part of the season. Since this in general is the period when the lowest minimum temperatures were experienced, with a consequent greater increase from the minimum to the temperature at which the respiration was determined, perhaps a greater stimulation could be expected. From a review of the literature it appears extremely doubtful whether the fruit used in these experiments, if stimulated at all by change in temperature, was stimulated more than can be explained by the dissolved carbon dioxide in the cell sap. The results with potatoes where increased metabolic activity is probably connected with the transformation of sugar to starch may not be applicable to apples. At any rate it would be hard to explain satisfactorily the rise in respiration late in the season on the basis of stimulation due to increase in temperature.

The decrease in oxidase activity during the growing season is in agreement with the writers' results with Bartlett pears (4) and with

the results of Hinton (11) with apples. The catalase activity of apples increased during the growing period in contrast to that of pears, for which the values formed a U-shaped curve (4). If there is high catalase activity in apples when the fruits are very small, it drops off much earlier than it does in Bartlett pears, for there was no indication of it in these studies. The results obtained in the present work offer additional evidence that oxidase and catalase activity are not directly correlated with respiration or with each other (5).

Scald or soft scald of apples did not occur in the lots used in these experiments, so no direct conclusions can be drawn as to the relation of rate of respiration and enzyme activity at harvest to their development in storage. Since the respiration and enzymatic curves are so similar in varieties subject to soft scald and in those that are not, it appears doubtful whether the susceptibility of different varieties is due to differences in the rate of respiration or in oxidase or catalase activity at harvest.

#### SUMMARY

The effect of maturity on the rate of respiration and on the oxidase and the catalase activity of apples has been studied.

Respiration decreased during the early part of the growing season and reached a minimum prior to the commercial harvesting of the fruit. The time intervening between harvest and the measurement of the respired carbon dioxide may determine whether or not a later increase is shown.

Oxidase activity decreased during the growing season and may or may not increase if the fruit is left on the tree past the normal harvest season.

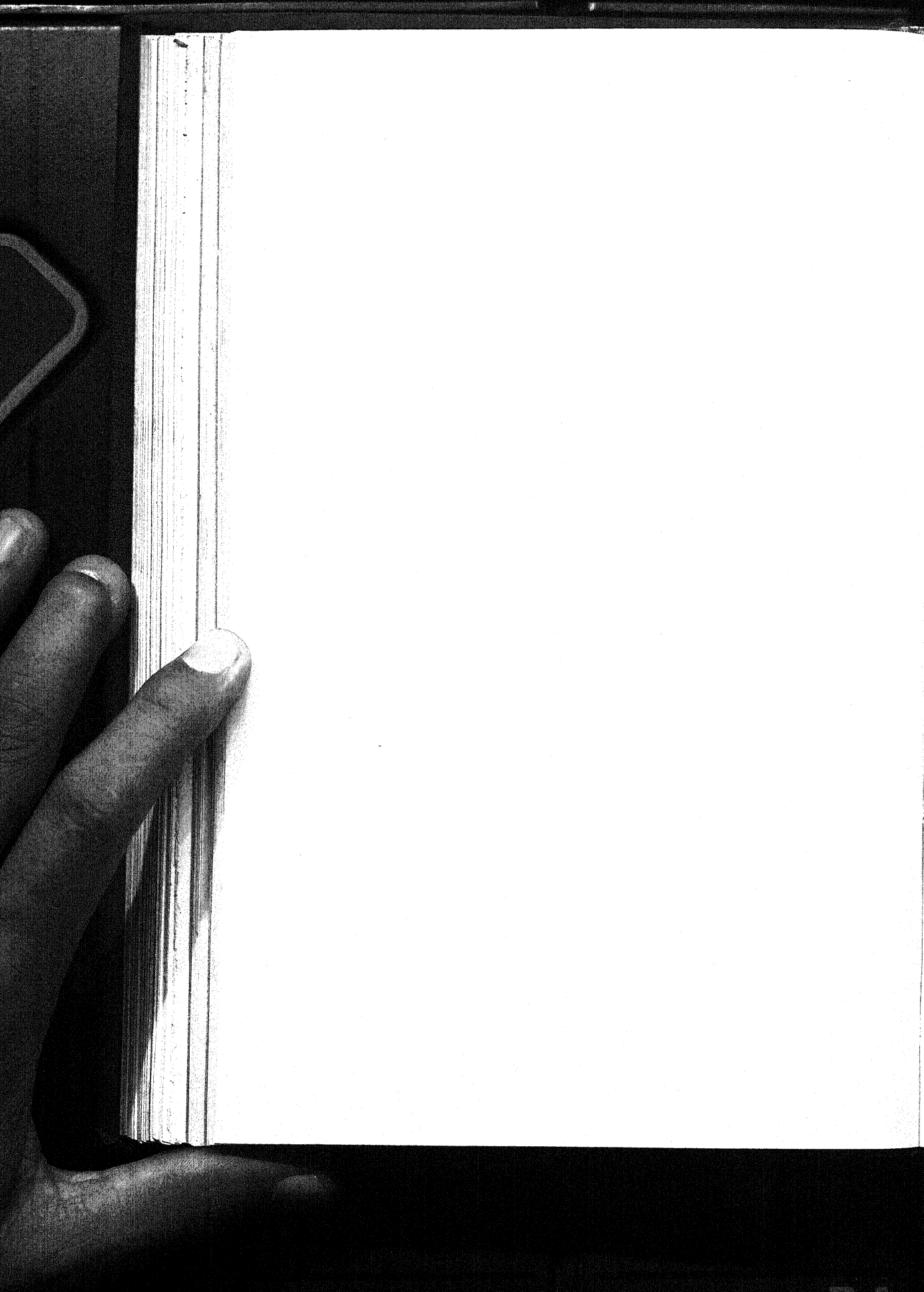
Catalase activity increased throughout the period from July to November.

Fruit harvested when fully mature usually showed a higher respiratory activity, a higher catalase activity, and a lower oxidase activity in storage than did fruit picked when less mature.

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# FACTORS DETERMINING THE REDUCTION IN YIELD OF FIELD CORN BY THE EUROPEAN CORN BORER<sup>1</sup>

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## INTRODUCTION

With the development of severe infestations of the European corn borer (*Pyrausta nubilalis* (Hbn.)) in the cornfields of Canada and the United States, questions arose regarding the reduction in yield resulting from definite populations of borers and also regarding the effect of plant development at the time of attack. The type and fertility of the soil were other factors that required consideration. All this information was needed as a guide in measuring crop losses and developing and interpreting control methods. It was also important to determine whether strains of corn (*Zea mays* L.) exist that will maintain their yields in the presence of a given population of borers to a greater degree than other strains of equal yielding capacity. Incidental to the study of these primary factors the data obtained provided an opportunity for studying the effect of the normal yield and the weather on borer damage.

The relation between type and fertility of the soil and yield of corn at definite borer populations was studied in northwestern Ohio with the variety Clarage from 1929 through 1933. The relation of plant development at the time of infestation and of the strain of corn to reduction in yield were studied primarily with hybrids and open-pollinated strains of corn at Sandusky and Toledo, Ohio, from 1930 through 1934.

## METHODS

Several methods for determining the reduction in yield of plants containing different numbers of borers were tried. One method depended on obtaining the average yields of groups of plants containing the same number of borers in each plant and then comparing these yields with those produced by uninfested plants of the same strain. However, the moths' habit of laying more eggs on the taller, thriftier plants, which gave greater yields, and the higher rate of borer survival on such plants were complicating factors. Finally, the use of average yields and borer populations from plants in replicated plots was found to be the desired method. Different borer populations were induced by infesting the plants by hand with egg masses produced

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<sup>2</sup> The authors are indebted to members of the staff of the Ohio Agricultural Experiment Station for providing the seed of Clarage corn and a plot of ground at Holgate, Ohio, in 1932 and 1933. G. W. Conrey, J. S. Cutler, and J. B. McLaughlin, of the Station staff, assisted in the study by selecting the soil types, conferring with the authors during the progress of the work, and making equipment available for drying and shelling the samples. J. R. Holbert, formerly of the Bureau of Plant Industry, U. S. Department of Agriculture, furnished the seed of most of the hybrids. P. T. Ullman, of the Division of Entomology, Indiana Department of Conservation, provided a location for an experiment in 1933. The authors are indebted also to the following members of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture: D. J. Caffrey and W. A. Baker, for general supervision; and B. A. App, C. A. Crooks, and R. T. Everly, for assistance with the field work.

in the laboratory according to methods described by Patch and Peirce.<sup>3</sup> The eggs hatched within a day of their placement on the plants. The date of hatching was about 9 days later than the average date for eggs laid naturally. This delay is believed to have reduced borer damage, for it is shown later that the reduction in yield of plants becomes less as the stage of development at this time becomes more advanced.

Each replicated plot was divided into as many equal subplots as there were borer levels planned for in the experiment. Each subplot, except one left for natural infestation, was infested with a definite number of egg masses per plant, the number depending on the borer population desired. To keep newly hatched larvae from being wind-blown from higher to lower infestation levels, the infestations in the subplots decreased from left to right in the row of plots on the left side of the experimental field, but increased from left to right in the row of plots next to the right, and this alternation was repeated across the field. Prior to the migration of many full-fed borers in August, a sample of plants from each subplot was dissected to determine the mean number of borers per plant for each population level. The number and size of the replicated plots, the number of population levels, and the size of the plant samples varied with the experiment. In most of the tests with the Clarage variety 5 population levels were replicated 3 or more times, from 30 to 50 plants per population level were dissected to determine the mean borer populations, and about 300 plants per borer level remained for yield determination. In the tests with the hybrids samples of 150 plants were adequate for the determination of yields. The yields were calculated in bushels of shelled corn per acre on the basis of a moisture content of 15.5 percent.

#### RELATION BETWEEN YIELD OF CORN AND BORER POPULATION

In areas heavily infested with the borer, it appeared to some observers that a slight reduction in yield due to the borers occurred in fields infested with less than about 5 borers per plant and that increasingly greater damage was caused as the borer population increased above this level. To determine whether the regression of yield on borers was linear or curvilinear, the average yields for the hybrids for the years 1930, 1931, and 1932 were plotted against the different borer populations. Yields for Clarage were also plotted against borer populations, but in this case the results for each of 3 degrees of soil fertility were averaged for the entire period 1929-32. From a supplementary experiment with Clarage and Smoky Dent in 1931 and 1932, data for various population levels up to 22.5 borers per plant were available. Inspection of each year's data obtained from Clarage and Smoky Dent indicated a linear relationship, which justified their combination for graphical presentation in figure 1. In the case of the hybrids, however, the data from the individual hybrids for each year were not extensive enough to warrant a test of linearity, but a grouping of all hybrids for each year, as was done for figure 1, indicates a linear relationship. The conclusion may be made, therefore, that in the case of Clarage and Smoky Dent, and possibly in the case of the hybrids, the reduction in the yield of corn was proportional to the number of corn borers per plant.

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## EFFECT OF NORMAL YIELD

While the effect of plant development at the time of borer attack and the effect of soil type and fertility on borer damage were being studied, it became apparent that direct comparisons were possible only when the estimated normal yields in the absence of borers were about the same. For this reason the effect of level of yield is discussed first.

The direct effect of yield level on the yield reduction per borer per plant was first observed on plantings of 24 hybrids at Sandusky and Toledo, Ohio. Five plantings were selected for study. Since in one planting the hybrids yielded from 57 to 105 bushels per acre and in

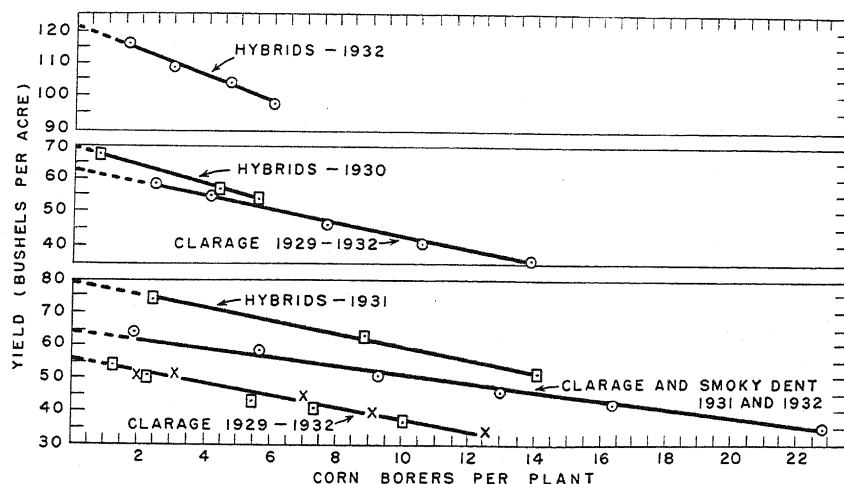


FIGURE 1.—Relationship between yield of corn and level of borer population induced experimentally. In the case of Clarage, 1929-32, plotted data from three levels of soil fertility are shown as follows: No fertilizer by squares, a single application of fertilizer by crosses, and a double application of fertilizer by circles.

another they yielded from 85 to 140 bushels, each planting included the range from 85 to 105 bushels. The average rate of yield reduction ( $B$ ) in bushels per acre for each unit increase in borers and the estimated yield in the absence of borers ( $A$ ) were calculated for each hybrid. In almost all cases the lowest population level was approximately 1 or 2 borers per plant; hence these estimates could be made with some confidence. The regression of  $B$  on  $A$  was then calculated for each planting (table 1). With the mean regression coefficient ( $+0.078 \pm 0.0129$ ), the mean yield of the five plantings ( $A=99.3$  bushels), and the mean yield reduction per borer per plant ( $B=3.69$  bushels), the calculated yield reduction ( $B'$ ) was found to average 2.57 bushels when  $A$  was 85 bushels and 4.13 bushels when  $A$  was 105 bushels. Expressed in percentages, the rates of yield reduction were 3.02 and 3.93 percent when the normal yields were 85 and 105 bushels, respectively.

TABLE 1.—Regression of yield reduction per unit of borer infestation (*B*) on the estimated yield of hybrid field corn in the absence of borers (*A*), Sandusky and Toledo, Ohio, 1931–34

Planting date	Hybrids <sup>1</sup>	Average yield per acre in absence of borers ( <i>A</i> )	Average per-acre yield reduction per borer per plant ( <i>B</i> )	Regression of <i>B</i> on <i>A</i>
	Number	Bushels	Bushels	Bushels
May 25, 1931.....	15	82.0	2.97	+0.052±0.0197
May 13, 1932.....	22	120.8	4.05	+0.070±0.0136
May 26, 1933 <sup>2</sup> .....	11	99.3	3.30	+0.058±0.0205
May 17, 1934.....	17	96.6	3.61	+0.095±0.0413
May 31, 1934.....	18	97.9	4.53	+0.115±0.0384
Mean.....		99.3	3.69	+0.078±0.0129

<sup>1</sup> Hybrids having a statistically significant value for *B* were used.<sup>2</sup> Since the mean values of *A* and *B* for the May 19 and June 2 plantings were nearly the same, data from both plantings were considered as one set.

Studies of the relation between level of yield and unit reduction in yield due to the borer under variable conditions of weather and soil fertility were made also on plantings of the Wooster strain of Clarage corn in several localities in northwestern Ohio from 1929 to 1933. The data for 18 of these plantings, including the estimated reduction in yield on the basis of regression, are given in table 2.

TABLE 2.—Reduction in yield of shelled corn of the Clarage variety by the European corn borer, in various plantings in northwestern Ohio, 1929–33; plantings arranged in order of increasing yield

Locality	Planting date	Estimated yield per acre in absence of borers ( <i>A</i> )	Reduction in yield per acre per borer per plant ( <i>B</i> )	Estimated reduction in yield per acre on basis of regression ( <i>B'</i> )	Range in borer population per plant
		Bushels	Bushels	Bushels	Numbers
Sandusky <sup>1</sup> .....	May 13, 1929.....	28.2	1.6±0.35	1.37±0.204	1.2–5.3
Holgate.....	May 26, 1933 <sup>2</sup> .....	40.8	1.7±.18	1.57±.137	.3–6.6
Huron <sup>1</sup> .....	May 15, 1929.....	42.4	1.2±.25	1.59±.130	1.6–9.2
Toledo.....	May 10, 1932.....	42.4	1.5±.10	1.59±.130	1.6–8.0
Huron <sup>1</sup> .....	May 9, 1930.....	43.5	1.8±.14	1.61±.126	1.1–5.8
	May 7, 1931.....	43.7	2.0±.02	1.62±.125	2.3–13.7
Maumee.....	May 11, 1932.....	45.5	1.6±.22	1.64±.118	1.3–10.1
	May 26, 1933 <sup>2</sup> .....	48.1	4.4±.29	1.69±.110	.6–4.4
Toledo.....	May 17, 1929.....	50.4	1.0±.04	1.72±.105	5.8–32.1
	May 13, 1931 <sup>1</sup> .....	52.5	1.7±.16	1.76±.103	.4–9.9
Sandusky <sup>1</sup> .....	May 7, 1930.....	53.8	2.5±.19	1.78±.102	1.3–6.1
Toledo.....	May 6, 1931.....	55.0	1.0±.14	1.80±.102	1.0–27.3
Holgate.....	May 1, 1932.....	56.8	2.1±.47	1.83±.103	1.4–8.0
Toledo.....	May 11, 1932.....	59.4	1.8±.19	1.87±.107	2.4–11.4
Sandusky <sup>1</sup> .....	May 6, 1931.....	66.5	1.9±.08	1.98±.130	2.6–19.3
	May 26, 1933 <sup>2</sup> .....	80.4	2.8±.35	2.20±.201	3.2–14.4
Toledo.....	May 5, 1932.....	80.8	1.9±.24	2.21±.204	2.0–22.7
Sandusky <sup>1</sup> .....	May 17, 1932.....	84.6	2.3±.22	2.27±.226	2.7–8.9
Mean.....	May 13.....	54.5	1.79		1.8–12.4

<sup>1</sup> Average of 3 levels of soil fertility.<sup>2</sup> Average of 2 plantings made on May 19 and June 2.<sup>3</sup> Not included in calculating the regression coefficient.

From the values in table 2 the regression of *B* on *A* was calculated. With 15 degrees of freedom the regression coefficient,  $0.016 \pm 0.0067$  bushel, may be regarded as significant. With this regression coefficient, the mean yield of the 17 plantings ( $A=54.5$  bushels), and the mean yield reduction per borer per plant ( $B=1.79$  bushels), it was calculated that  $B'$  would average 1.37 bushels when *A* was 28.2



bushels and 2.27 bushels when  $A$  was 84.6 bushels. Expressed in percentages the rates of yield reduction were 4.86 and 2.68 percent when the normal yields were 28.2 and 84.6 bushels, respectively. The estimated reduction in yield on the basis of regression was also calculated for each planting of Clarage listed in table 2.

Before comparisons are made between the data from Clarage and from the hybrids, the conditions under which the two sets of data were obtained should be examined. Since the reduction in yield due to the borer increases as the stage of plant development at the time of borer hatch becomes less advanced, the question arises whether the greater unit reduction in yield for the hybrids at the highest level was directly associated with increase in the level of yield or was caused by greater damage to later silking hybrids that yielded more. In the five plantings under consideration (table 1), on an average 82.6 percent of the hybrids silked within 3 days, and on one planting in each of 3 years 13, 14, and 11 hybrids, respectively, silked within 2 days. For hybrids having the same or the succeeding day as their average silking date, it was determined that for each planting the regression of  $B$  on  $A$  was significant. As an average of these 3 plantings, for each unit increase in the normal yield the yield reduction per borer per plant increased  $0.072 \pm 0.0154$  bushel as compared with 0.078 bushel, the average value for the five plantings. The generally narrow range in silking dates and the highly significant regression of  $B$  on  $A$  obtained for the hybrids silking within 2 days indicate that greater damage per borer at higher levels of yield was directly associated with the increase in the level of yield in the plantings considered.

In the case of Clarage there were only slight differences in plant development at time of infestation between the plantings giving the largest and the smallest yields. No reason is known why the greater unit reduction in yield at the high yield level may not be considered as being also directly associated with increase in the level of yield.

From a study of the data from Clarage and the hybrids the following comparisons are noteworthy:

(1) On the basis of a purely mathematical relationship a constant percentage of the crop would be expected at all yield levels. In Clarage, however, the loss was found to be 4.86 percent of the yield per borer per plant at the yield level of 28.2 bushels and 2.68 percent at the yield level of 84.6 bushels. Evidently there were factors involved to change the expected relationship. It is possible that the uninjured tissue within the stalk is progressively able to do more of the work of the injured tissue as the plant's environment enables it to yield more. As a matter of fact, with hybrids a given number of borers were found to reduce the yield of plants in the same field only about half as much in the relatively wet season of 1931 as in the 1930 season of drought.

(2) In Clarage the percentage reduction in yield was considerably greater at the lowest level of yield than at the highest level, whereas in the hybrids the percentage reduction was greater at the highest level of yield. The reason for this reversal in trend is not known.

(3) At the yield level of 85 bushels,  $B'$  was calculated to be  $3.02 \pm 0.249$  percent per borer per plant for the hybrids and  $2.68 \pm 0.268$  percent for Clarage. The difference is not significant. It is possible, however, that some of the difference in favor of Clarage may have

been due to the difference in planting date, which averaged 9 days earlier for Clarage. At the time of infestation Clarage was in a more advanced stage of development than the hybrids. Another study<sup>4</sup> showed that hybrids were more tolerant to the corn borer than open-pollinated varieties in the same plantings, the percentage of yield reduction being less for the hybrids. In the present study it is impossible to compare the hybrids and open-pollinated Clarage for relative tolerance.

#### EFFECT OF WEATHER

In the planting of Clarage made on May 7, 1930, at Sandusky (table 2), the difference between the observed and the predicted rate of yield reduction may be regarded as highly significant. The drought of 1930 is suggested as an explanation for the high observed rate. The rainfall from 6 showers during the critical period of growth, from June 20 to August 20, totaled only 1.83 inches with a maximum of 0.60 inch, and the temperature averaged 2.2°, 1.8°, and 1° F. above normal for June, July, and August, respectively. Moreover, in another field on the same farm 28 hybrids and open-pollinated varieties also showed a high yield reduction per borer per plant. The hybrids and open-pollinated varieties were planted a day earlier than Clarage. Their estimated average normal yield of 69.3 bushels was reduced on an average  $2.9 \pm 0.11$  bushels per borer per plant as compared with an average reduction of  $1.5 \pm 0.14$  bushels from 14 strains planted in the same field on May 5 and 12, 1931, on which the normal yield averaged 70 bushels per acre. From June 20 to August 20, 1931, 18 showers of more than 0.04 inch rainfall totaled 7.60 inches with maxima of 0.65, 0.35, and 1.68 inches in June, July, and August, respectively. The temperature averaged 1.0°, 4.6°, and 2.1° above normal in the 3 months. The corn-growing season of 1931 was therefore more favorable than that of 1930, for only about half as much yield reduction per borer occurred in strains planted in the same field and having about the same level of yield.

The rates of reduction in yield due to the borer for the 1931 planting at Huron and especially for the 1933 planting at Maumee were also significantly greater than those predicted on the basis of normal yield (table 2). A planting in 1932 at the Maumee location showed little difference between observed and estimated reduction in yield. The level of yield was the same, but the field was planted earlier than in 1933. Although weather records were not taken at the Maumee location, the 1933 season was in general drier than the 1932 season. Dry conditions may have contributed to the high rate of borer damage on this planting as well as at Sandusky in 1930.

#### EFFECT OF SOIL FERTILITY

In eight of the plantings of Clarage the experiment was conducted at three levels of soil fertility—(1) on soil receiving no fertilizer, (2) on soil receiving a unit application of fertilizer, and (3) on soil receiving twice as much as (2). The fertilizer varied in amount and formula with the soil type. The differences in yield reduction per borer per plant between the three fertility levels were not significant

<sup>4</sup> PATCH, L. H., STILL, G. W., APP, B. A., and CROOKS, C. A. COMPARATIVE INJURY BY THE EUROPEAN CORN BORER TO OPEN-POLLINATED AND HYBRID FIELD CORN. *Jour. Agr. Res.* 63: 355-368. 1941.

in most of the plantings. When averaged for the eight plantings, the estimated yield of 48.3 bushels per acre in the absence of borers was reduced  $2.0 \pm 0.17$  bushels per borer per plant where no fertilizer was applied, the yield of 50.7 bushels was reduced  $1.7 \pm 0.10$  bushels where the unit amount of fertilizer was applied, and the yield of 56.8 bushels was reduced  $1.9 \pm 0.09$  bushels where twice the amount of fertilizer was applied. Since the differences in yield reduction are not statistically significant, and the differences among the normal yields are not large, it appears that the fertility of the soil had little effect, if any, on the damage by the corn borer in these tests.

#### EFFECT OF TYPE OF SOIL

In the plantings listed in table 2 the type of soil also varied. At Huron the field was a clay loam derived from shale-sandstone, at Sandusky it was a very fine sandy loam, at one location in Toledo the soil was a very fine sandy loam and at another silty clay, at Maumee the soil was light-colored Fulton silt loam, and at Holgate it was black Brockston clay. On the very fine sandy loam at Sandusky in 1929, 1930, and 1931 the average estimated yield of Clarage in the absence of borers was about 49.7 bushels per acre; the corn borer reduced this yield at the average rate of  $2.0 \pm 0.10$  bushels per borer per plant. On the clay loam from shale-sandstone at Huron the average yield of 43.8 bushels for the same years was reduced at the rate of  $1.7 \pm 0.11$  bushels per borer per plant. The difference between the rates of yield reduction on these two types of soil is only what might be expected if the rate of reduction is considered a function of the level of normal yield. Other comparisons between types of soil furnished no evidence that any factors other than level of yield are involved.

#### EFFECT OF STAGE OF PLANT DEVELOPMENT

In studies on the effect of stage of plant development at the time of borer hatch on yield reduction due to the corn borer, the interval between the hatching of the borers and the silking of the corn was varied (1) by infesting samples of plants of the same planting on different dates and (2) by infesting samples of different plantings on the same date.

In 1932 an experiment was conducted to determine the rate of yield reduction of plants infested at different times. Two open-pollinated varieties and four hybrids were planted on May 16 in 8-hill plots replicated 15 times. Four borer levels were used, the plants were infested on 3 dates, and the yield was based on a total of about 160 plants from the 6 strains of corn for each borer level of plants infested on each date. The average date of silking was July 26, and in the absence of borers the estimated yield should have averaged 84.1 bushels per acre. This potential yield was reduced  $3.5 \pm 0.85$  bushels per borer per plant when the plants were infested on June 27,  $2.8 \pm 0.53$  bushels when infested on July 8, and  $1.9 \pm 0.31$  bushels when infested on July 18.

Although the earliest infested plots showed the greatest reduction in yield, the differences between the rates were not found to be significant. It is probable, however, that larger samples of plants,

or more borer levels, would have reduced the variability of the data sufficiently to show significance, since data derived from other sources show a significant trend of change in the rate of reduction of yield associated with stage of plant development.

The rates of yield reduction in various plantings in which the infestation date for the plantings within a season was uniform are given in table 3. Since the mean levels of yield for the earliest and latest plantings differ by less than a bushel, the rates of yield reduction are directly comparable. The difference of  $1.6 \pm 0.30$  bushels may be regarded as highly significant with the 156 degrees of freedom available. This difference is associated with the difference of 23 days between the average dates of the earliest and latest plantings, and an interval of 11 days from egg hatching to corn silking. The yield reduction per borer was 2.85 percent of the normal yield of the earliest plantings as compared with 4.71 percent for plantings made 23 days later. An average of five borers per plant in each of the earliest plantings would have reduced the yield nearly as much as an average of three borers per plant in each of the latest plantings, because a larger percentage of the late-planted crops was destroyed by the borer. It is probable that the plants of the late plantings infested early in their development were subject to greater reductions in yield, owing to the longer duration of borer feeding before the critical period of ear production and the consequent weaker condition of the plants and the larger average size of the borers during the period of ear production.

TABLE 3.—Reduction in yield of shelled corn by the European corn borer, Ohio and Indiana, 1931-34

Locality	Date of planting	Mean date of silking	Period from egg hatching to corn silking	Estimated yield per acre in absence of borers	Observed reduction in yield per acre per borer per plant	Degrees of freedom
	1931		Days	Bushels	Bushels	
Sandusky, Ohio.....	May 5	July 27	11	84.8	$1.6 \pm 0.10$	24
	May 12	July 28	12	80.2	$1.7 \pm .10$	24
	May 19	July 31	15	74.9	$2.2 \pm .21$	24
	May 25	Aug. 1	16	76.5	$2.5 \pm .21$	24
	1932					
	May 7	July 14	9	94.0	$2.9 \pm .46$	11
	June 7	Aug. 2	26	96.7	$6.1 \pm 1.00$	11
Toledo, Maumee, and Holgate, Ohio, and Auburn, Ind. <sup>1</sup> .....	1933					
	May 19	July 30	16	67.1	$2.7 \pm .10$	19
	June 2	Aug. 5	22	71.9	$3.0 \pm .22$	19
Toledo, Ohio.....	1934					
	May 3	July 17	9	91.1	$2.3 \pm .12$	24
	May 17	July 25	17	91.4	$3.3 \pm .17$	24
	May 31	Aug. 2	25	94.9	$4.3 \pm .19$	24
Mean for period 1931-34:						
Earliest planting.....	May 9	July 22	11	84.3	$2.4 \pm .12$	11
Latest planting.....	June 1	Aug. 3	22	85.0	$4.0 \pm .27$	22

<sup>1</sup> Data are averages from the 4 localities.

#### EFFECT OF STRAIN OF CORN

The data from the hybrids obtained over the period 1930-34 were used to test the significance of the deviation of each hybrid from the regression line of *B* on *A*. The basic data were the same as those used for calculating the data in table 1. The observed rate of yield reduc-



tion, *B*, was found to be significantly less than the rate predicted on the basis of regression on *A* in only 4 out of 149 tests, 2 of which were of the single-cross hybrid III. A  $\times$  Ind. TR. Apparently few hybrids are able to maintain their yield in the presence of a given number of borers to a greater degree than other hybrids of equal yielding capacity.

The yield of hybrid III. A  $\times$  Ind. TR should have been reduced 3.67 bushels per acre for each additional borer per plant had it reacted to the borer according to prediction. Actually its yield was reduced on an average 2.35 bushels per borer per plant. Patch and others<sup>5 6</sup> have found, however, that the survival of borers on this hybrid is higher than the average. Hence, any advantage from having smaller losses in yield per borer is partly, if not wholly, offset by the greater than average number of borers to cause reductions in yields.

In 1931 and 1932 a small-stalked early variety, Smoky Dent, and a medium-stalked midseason variety, Clarage, were compared with each other and with a large-stalked late variety, Johnson County White. The interval from planting to silking averaged 71, 78, and 89 days, and the height of the plants at maturity averaged 90, 103, and 108 inches, respectively. The peak of hand infestation was 15 days later than the peak of natural infestation in 1931 and 14 days later in 1932. In another experiment with the same varieties in 1933 the diameter of the plants averaged 0.81, 0.83, and 1.02 inches at the second internode.

As an average of the tests of 1931 and 1932, the normal yield of Smoky Dent of 63.6 bushels was reduced  $1.3 \pm 0.06$  bushels per borer per plant, the yield of Clarage of 67.9 bushels was reduced  $1.5 \pm 0.14$  bushels, and the yield of Johnson County White of 79.1 bushels was reduced  $2.2 \pm 0.21$  bushels. These rates of yield reduction are lower than the rates expected for the yield levels indicated, probably because the plants were infested later than usual. The small difference in the rate of yield reduction between Smoky Dent and Clarage is not significant. The difference of 4.3 bushels between the normal yields of the two strains is also small. A greater reduction in yield that possibly might have been expected on account of the smallness of Smoky Dent might well have been offset by the decrease due to its earliness, for the interval between borer hatch and the beginning of ear development would have been shortened, and consequently its plants were probably in a less weakened condition than were those of Clarage during the period of ear development.

Johnson County White had an average yield of 11.2 more bushels per acre than Clarage, and it silked 11 days later. Its yield was reduced at a significantly greater rate than the yield of Clarage. A decreased rate of yield reduction that possibly might have been expected on account of the large size of Johnson County White might well have been offset by the increase due to its lateness and higher level of yield. Because of its lateness Johnson County White would have been in a more vulnerable condition than Clarage during the period of ear development.

Neiswander and Herr<sup>7</sup> also studied the reduction in yield of early and later maturing strains infested by the corn borer. Using Burr

<sup>5</sup> PATCH, L. H. RESISTANCE OF A SINGLE-CROSS HYBRID STRAIN OF FIELD CORN TO EUROPEAN CORN BORER. *Jour. Econ. Ent.* 30: 271-278. 1937.

<sup>6</sup> PATCH, L. H., BOTTGER, G. T., and APP, B. A. COMPARATIVE RESISTANCE TO THE EUROPEAN CORN BORER OF TWO HYBRID STRAINS OF FIELD CORN AT TOLEDO, OHIO. *Jour. Econ. Ent.* 31: 337-340. 1938.

<sup>7</sup> NEISWANDER, C. R., and HERR, E. A. CORRELATION OF CORN BORER POPULATION WITH DEGREE OF DAMAGE. *Jour. Econ. Ent.* 23: 938-945, illus. 1930.

Leaming and Smoky Dent, silking 6.7 days earlier than Burr Leaming, as two field-corn varieties, and Golden Bantam, a sweet-corn variety silking 4.1 days earlier than Smoky Dent, they concluded that their data indicated "considerable variation in the damage resulting to different varieties from a given borer population, the amount of damage per borer increasing as the length of growing season of the variety decreases." Comparisons were made on the basis of yields taken after the ears of the field-corn varieties were mature and while Golden Bantam was in the roasting-ear stage. In the present study of field corn no evidence was found to support their conclusion. Other studies indicate that field- and sweet-corn varieties are not directly comparable as to damage by the corn borer.

#### SUMMARY

The degree of reduction in yield of field corn resulting from definite levels of population of the European corn borer was determined by manually infesting plants with various numbers of egg masses. Reduction in yield within cornfields is shown to be proportional to the number of borers present up to 22 borers per plant. Within the range from 28 to 85 bushels per acre the fields that would have produced greater normal yields in the absence of borers had smaller percentages of their crops destroyed by a given number of borers than the fields with lower yields. Data from plantings of the Clarage variety in various localities in northwestern Ohio from 1929 to 1933 showed that the rates of yield reduction were 2.68 and 4.86 percent per borer per plant when the normal yields were 85 and 28 bushels per acre, respectively. The rate of yield reduction for the hybrids was 3.93 percent per borer per plant when the normal yield was 105 bushels per acre as compared with 3.02 percent when the normal yield was 85 bushels.

The rate of yield reduction per borer in corn planted on soils differing in type or fertility is probably a direct function of the level of normal yield. In seasons of drought the damage per borer at given yield levels appears to be increased.

Stage of plant development at time of infestation is shown to be an important factor in yield reduction in the presence of the borer. Plants infested early in their development suffered greater rates of yield reduction, owing to the longer duration of borer feeding before the critical period of ear production and the consequent weaker condition of the plants, and to the larger average size of the borers during the period of ear production. The average normal yield of 85 bushels per acre over a 4-year period, for corn planted on the average date May 9, was reduced 2.85 percent per borer as compared with 4.71 percent for the same hybrids giving about the same average yield but planted 23 days later. An average of five borers per plant in each of the earliest plantings, therefore, would have reduced the yield nearly as much as an average of three borers per plant in each of the latest plantings.

Attempts were made, without much success, to find hybrids having the ability to maintain their yields in the presence of a given level of borer population to a greater degree than would other hybrids of equal yielding ability.

## HYDROCEPHALUS, A LETHAL IN CATTLE<sup>1</sup>

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### INTRODUCTION

Hydrocephalus has been reported as an inherited lethal in swine (1)<sup>2</sup> and mice (2, 4). Many sporadic cases have been reported in man, cattle, chickens, dogs, and rats. There are two types variously reported: (1) Internal hydrocephalus, a collection of fluid in the cerebral ventricles, and (2) external hydrocephalus, a collection of fluid outside the brain substance.

Houck (7) reported two cases of congenital hydrocephalus, one a grade Durham calf and the other an albino rat. The condition was not lethal in either instance. In the rat, however, blindness was associated with hydrocephalus and the animal eventually died from starvation.

Clark (2) has noted hereditary hydrocephalus in the house mouse which appeared in the F<sub>2</sub> and backcross generations of mice with the flexed-tail character. It was apparently due to a single Mendelian factor, the homozygous recessive being lethal. Frequent association with the flexed-tail character suggested linkage or another manifestation of the gene for flexed (2). Later work (3) showed that hydrocephalus was not caused by the same gene as flexed tail. Still further experiments (4) showed no linkage between hydrocephalus and various other characters, including flexed tail.

Blunn and Hughes (1) have recorded a lethal hydrocephalus of the external type in Duroc-Jersey swine, the fluid being found outside the brain in the subarachnoid spaces. Varying degrees of hydrocephalus occurred, but a short tail or no tail always accompanied the defect regardless of its degree. Light coat color also accompanied the defect in all except 3 cases. All the affected pigs were the result of inbred matings. Twenty litters from heterozygous parents produced 178 animals, of which 42 were hydrocephalic and 136 normal. It was suggested that the three associated characters might be the manifold effects of one factor, or they might be caused by very closely linked genes. The goodness of fit between the observed and expected 3 : 1 ratio ( $\chi^2=0.187$ .  $P$ =between 0.5 and 0.7) indicates that the hydrocephalus syndrome was caused by a single recessive autosomal gene.

Hyde (8) observed an epidemic of hydrocephalus in experimental rabbits, but was unable to offer any rational explanation for its occurrence or disappearance.

Ely, Hull, and Morrison (6) reported one case of slight hydrocephalus among four cases of agnathia in Jersey calves.

Morrill (10) observed that hydrocephalus occurs in certain breeds of dogs, notably in the German boxer, and he recorded occasional

<sup>1</sup> Received for publication February 5, 1942.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 490.

observations in single individuals of other breeds. He studied several purebreds and various crosses. Among the purebreds, the British bull showed marked external hydrocephalus in four out of five cases examined; one case was practically normal. Among hybrids, the condition was most often seen in the great Dane  $\times$  St. Bernard crosses, but in varying degrees.

Schlotthauer (11) noted five random cases of internal hydrocephalus in dogs. In only one case was there any evidence that the condition was hereditary.

Eaton (5) summarized 15 known lethals in cattle. None of these corresponds in any way with the lethal reported here.

#### EXPERIMENTAL RESULTS AND OBSERVATIONS

The lethal to be described was discovered in a herd of grade and purebred Holstein-Friesian cattle. Purebred bulls had been used for several generations, but insofar as could be determined, no inbreeding had been practiced before 1938. Early in 1940 the owner interviewed one of the authors relative to a possible nutritional deficiency in his herd. In December 1939, an abnormal calf had been born but no particular significance was attached to the occurrence. However, when another calf with the same abnormalities was born in January 1940, the owner became worried about his ration. A careful check revealed that the herd had been fed legume roughage, mainly alfalfa hay with some sweetclover, and a grain mixture composed of corn and cob meal, oats, and soybean oil meal. The cattle were given free access to a mixture of 1 part bonemeal and 2 parts salt during the winter months, and salt alone during the pasture season. There seemed to be no reason for suspecting a nutritional deficiency and the two cases suggested something other than chance. A check on the breeding program revealed that the two heifers that had given birth to the abnormal calves had been mated to their sire. Previous to these two abnormalities, no unusual calves had been born except that in June 1939 a calf from a similar mating had shown a highly nervous condition, and was unable to stand alone. No improvement of this condition was observed and the calf was later vealed. In December 1939 and January 1940 two calves were born from sire-daughter matings that were normal in every way, except for asymmetrical faces. A similar "wry jaw" defect was reported by Lutikov (9) in a study of breeding methods practiced in a herd of Jaroslav cattle.

The breeding program in this herd suggested an inherited condition and so arrangement was made with the owner whereby an additional number of sire-daughter matings would be made. Twenty-seven calves were produced as a result of sire-daughter matings, involving 15 daughters. The results of these matings are given in table 1.

TABLE 1.—Date of calving, and condition and sex of calf from sire-daughter matings involving 15 daughters

Cow No.	Date of calving	Condition of calf	Sex
23.....	Nov. 24, 1938.....	Normal.....	Male.
	Jan. 23, 1940.....	Lethal.....	Do.
	Dec. 30, 1940.....	do.....	Do.
24.....	June 1, 1939.....	Jumpy.....	Female.
	May 8, 1940.....	Normal.....	Do.
	Apr. 21, 1941.....	do.....	Male.



TABLE 1.—*Date of calving, and condition and sex of calf from sire-daughter matings involving 15 daughters—Continued*

Cow No.	Date of calving	Condition of calf	Sex
25.....	June 26, 1939	Normal	Male.
26.....	May 16, 1940	do	Female.
27.....	Dec. 6, 1939	do	Male.
28.....	Nov. 3, 1940	do	Female.
29.....	Dec. 25, 1939	Lethal	Male.
30.....	Mar. 1, 1940 <sup>1</sup>	Normal fetus	Undetermined
31.....	Dec. 27, 1939	Asymmetrical	Female.
32.....	Jan. 31, 1941	Lethal	Male.
33.....	Dec. 27, 1939	Normal	Do.
34.....	Jan. 1, 1941	Lethal	Do.
35.....	Jan. 12, 1940	Asymmetrical	Female.
36.....	( <sup>2</sup> )	Normal fetus	Undetermined.
37.....	Feb. 4, 1940	Normal	Male.
38.....	Jan. 15, 1941	do	Do.
39.....	Mar. 22, 1940	do	Female.
40.....	Mar. 16, 1941	do	Do.
41.....	Dec. 22, 1940	do	Male.
42.....	Jan. 7, 1941	do	Female.
43.....	Feb. 7, 1941	Jumpy	Do.
44.....	May 21, 1941	Asymmetrical and lethal	Do.
45.....	June 12, 1941	Normal	Male.
Totals (15 cows, 27 calves).....		5 lethal..... 1 asymmetrical and lethal..... 2 asymmetrical..... 2 jumpy..... 17 normal.....	14 males. 11 females. 2 undetermined.

<sup>1</sup> Cow butchered in advanced pregnancy.<sup>2</sup> Cow died in advanced pregnancy.

The lethal was manifested by an internal hydrocephalus accompanied by a marked papilledema. The lateral ventricles were greatly distended so that only a thin layer of cerebral tissue remained between the cavity of the ventricles and the cranial bones. On dissection, no abnormalities were found which might suggest a lack of embryological development of the arachnoid villi, nor were there any apparent points of blockage in the various foramina so far as could be determined. As a result of the pressure developed, the cranial cavity of the skull was enlarged from two to three times the normal size, as shown in figures 1 and 2.

Ventrally the position and angle of the foramen magnum was markedly altered from that of a normal animal, as shown in figure 3. It is possible that this abnormality might partly or completely block the foramina of Magendie and Luschka, thereby causing the hydrocephalus as a secondary manifestation of a gene for bony abnormalities. Figure 3 shows also a marked widening of the space between the mandibles as compared to the normal.

Both the humeri and femurs of these animals showed marked malformation. The shaft of these bones was considerably shortened, but larger in diameter than the normal, as shown in figure 4.

The condyles and heads of both bones had the appearance of having had pressure applied against the articulating surfaces so that they were bent down toward the shaft and twisted to one side. Previous to dissection, the twisted condition of the femur resulted in extreme width through the hips, causing difficult parturition. The abnormal humerus also resulted in a twisted appearance of the forelegs (fig. 5).



FIGURE 1.—Frontal view of hydrocephalus calf, showing marked enlargement of the skull; note twisted condition of forelegs.

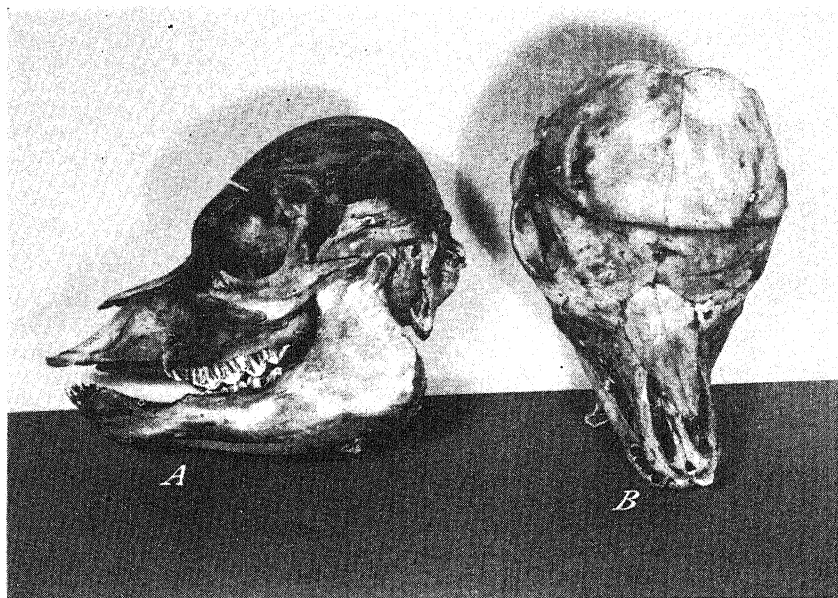


FIGURE 2.—Side view (A) and frontal view (B) of hydrocephalus skulls; B shows asymmetrical condition.

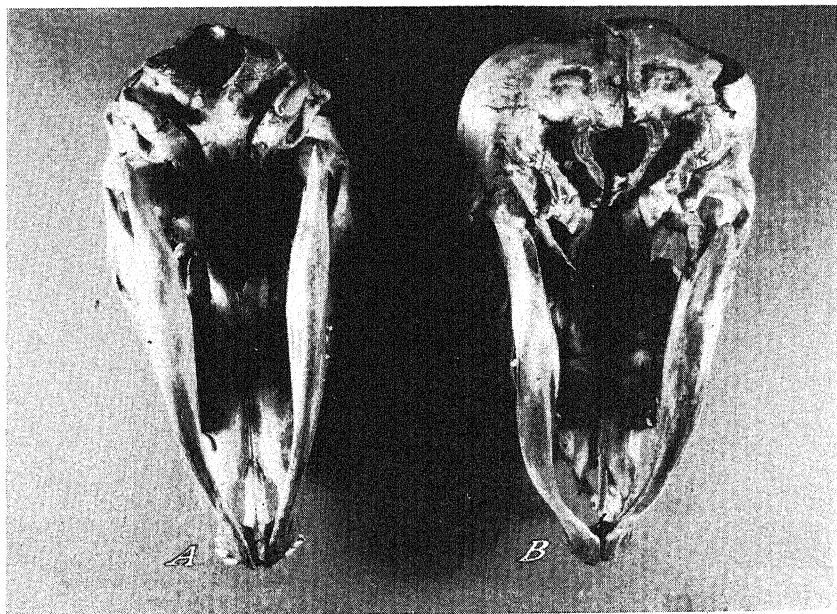


FIGURE 3.—View of skulls showing position of foramen magnum and also abnormal condition of mandibles: A, normal; B, lethal.



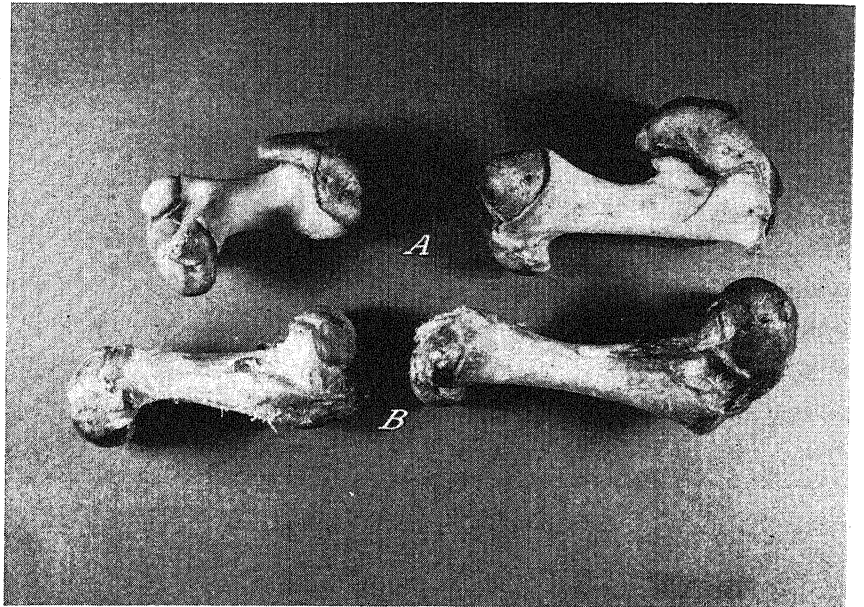


FIGURE 4.—Humerus and femur from an abnormal (*A*) and a normal calf (*B*).

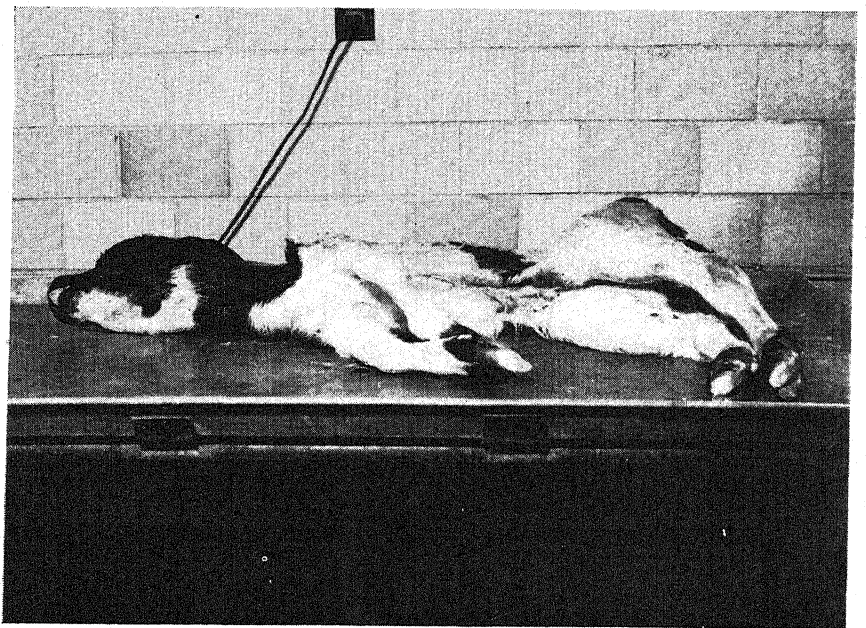


FIGURE 5.—View of abnormal calf showing extreme width of hips and twisted forelegs.



The asymmetry of the skull and face was shown most distinctly in the living animal by a twisting of the face to one side. When the head was cleaned of surrounding tissues, asymmetry of the lower jaw and facial bones was quite apparent. In addition, the bony structures of the cranium were unequally developed and displaced to one side of the midline. In the specimen photographed in figure 2, which is typical of these individuals, the unequal development of the cranial bones is well shown, as is the twisted appearance of the face. In addition, the left mandible was considerably shorter than the right. This was also true for the maxillae.

The condition designated as "jumpy" was suggestive of cerebellar or thalamic dysfunction. Marked lack of muscular coordination and control was at all times evident. These calves were unable to rise or stand unaided. When placed on their feet, the weight was supported reasonably well for a short time at least. In this position, however, the animal tended to sway from side to side or backward and forward and unless supported was unable to remain standing. Tremor was present in both the upright and lying positions. In the lying position, in addition to the tremor, there was almost constant movement of the limbs. Resistance to flexion of the limbs was present irregularly.

Possibly the lethal trait, the asymmetry, and "jumpy" are due to three recessive autosomal genes.

A genetic analysis based on the above hypothesis may now be attempted. The three pairs of alleles would be as follows:

<i>L</i> —normal	<i>l</i> —lethal
<i>A</i> —symmetrical	<i>a</i> —asymmetrical
<i>J</i> —normal nervous reaction	<i>j</i> —"jumpy"

The sire should have the genotype *LLAaJj*.

The daughters from normal dams would have eight different genotypes, as follows: *LLAAJJ*, *LLAAJj*, *LLAaJJ*, *LLAaJj*, *LLAAJj*, *LLAaJj*, and *LLAaJj*. These daughters backcrossed to their sire would then produce the following percentages of phenotypes in their offspring: *LAJ*, 66.992; *LAj*, 9.570; *LaJ*, 9.570; *LAJ*, 9.570; *LAj*, 1.367; *laJ*, 1.367; *LaJ*, 1.367; and *laj*, 0.195.

The expected and observed distribution of the 27 calves by phenotype would, therefore, be as shown in table 2. In this table *LAJ*

TABLE 2.—Expected and observed distribution of the 27 calves by phenotype

Distribution	<i>LAJ</i>	<i>LAj</i>	<i>LaJ</i>	<i>LAJ-lAj</i>	<i>Laj</i>	<i>laJ-laj</i>
Expected.....	18.09	2.58	2.58	2.95	3.37	0.42
Observed.....	17.0	2.0	2.0	5.	0	1.0
Difference.....	-1.09	-.58	-.58	+2.05	-.37	+.58

and *LAj* are combined as are also *laJ* and *laj*. These combinations must be made because of the impossibility of observing the "jumpy" condition in the "*l*" calves which are born dead. The goodness of fit between the observed and expected values ( $\chi^2 = 2.92$ ;  $P =$  between 0.7 and 0.8) suggests the action of three recessive nonlinked genes.

Breaking the trihybrid ratio down into three monohybrid ratios, the expected and observed values are as shown in table 3, which also shows the expected and observed incidence of the "jumpy" animals among the nonlethals.

TABLE 3.—*Expected and observed values for lethal-normal, asymmetrical-normal, and "jumpy"-normal animals*

Contrasted types	Ratio—		$\chi^2$ <sup>1</sup>	P
	Expected	Observed		
Lethal-normal.....	3.38 : 23.62	6 : 21	2.32	Between 0.2 and -0.1.
Asymmetrical-normal.....	3.38 : 23.62	3 : 24	.05	Between 0.9 and -0.8.
"Jumpy"-normal.....	2.63 : 18.37	2 : 19	.17	Between 0.7 and -0.5.

<sup>1</sup> Although the chi-square test has been used, the numbers are smaller than are desirable for such an analysis.

### CONCLUSIONS

A new lethal in cattle, internal hydrocephalus, is described and shown to be probably a simple recessive in its mode of inheritance.

Two other conditions, asymmetry and "jumpy," are described and may be recessive and not linked with each other or with the lethal gene.

The data suggest that the sire used carried three rare recessive genes. Nevertheless, the probability that any one animal would carry three rare factors is very small. However, the facts are stated and a conclusion indicated, though the mode of inheritance of the characters "jumpy" and asymmetry should be studied further.

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# DEVELOPMENT OF SYNTHETIC FOOD MEDIA FOR USE IN NUTRITION STUDIES OF THE EUROPEAN CORN BORER<sup>1</sup>

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## INTRODUCTION

Studies on the nutrition of the European corn borer (*Pyrausta nubilalis* (Hbn.)), since their initiation in 1937 at the Toledo, Ohio, research laboratory, have been directed principally toward finding chemical substances contained in green plant tissue, particularly corn (*Zea mays* L.), which are associated with the survival, growth, and metamorphosis of the insect. Preliminary studies reported by Bottger<sup>3</sup> and subsequent feeding tests, supplemented with chemical analyses of the test materials, besides adding appreciably to available information relative both to the insect and to the chemistry of various strains of corn, have emphasized a long-recognized need for a synthetic food medium that would facilitate a more comprehensive study of the nutritive requirements of the organism. Such a medium would eliminate the necessity of difficult, and often unsatisfactory, chemical analyses and would permit an accurate comparison of the effect of each nutritive constituent, including the vitamins, on the physiological development of the insect and its relation to borer-resistant strains of corn.

On the basis of information accumulated from various experiments, a series of tests was designed for the purpose of rearing corn borer larvae to maturity on a synthetic medium.

## METHODS, TEST MATERIAL, AND EQUIPMENT

Twenty synthetic food media were formulated to simulate roughly in carbohydrate, protein, and moisture content some samples of green corn tissue obtained through the cooperation of the Bureau of Plant Industry of the United States Department of Agriculture and the Ohio Agricultural Experiment Station. The mineral analyses of corn plants reported by Latshaw and Miller<sup>4</sup> served as a general guide to the minerals to be included, though no attempt was made to approximate the relative percentages of each element. Each medium was infested with 100 or more newly hatched corn borer larvae. A small quantity of food material was spread about ¼ inch thick around the side walls of glass crystallizing dishes, which served as rearing chambers. The rearing chambers were 2 inches in diameter by 1½ inches

<sup>1</sup> Received for publication March 18, 1942.

<sup>2</sup> Grateful acknowledgment of assistance is made to W. A. Baker, in charge of European corn borer research, under whose leadership these studies were initiated and conducted.

<sup>3</sup> BOTTGER, G. T. PRELIMINARY STUDIES OF THE NUTRITIVE REQUIREMENTS OF THE EUROPEAN CORN BORER. Jour. Agr. Res. 60: 249-257. 1940.

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deep, and covered with an 80-mesh copper-screen disk, which was held in place with a  $\frac{3}{4}$ -inch rubber band. The food in each dish was infested with 20 individuals, and the number was gradually reduced as growth of the larvae increased their space and food requirements. The larvae were transferred to fresh food and a clean receptacle every second or third day during a 40-day test period. After the tenth day all larvae were weighed individually when they were transferred to fresh food.

Numerous preliminary tests, utilizing various materials as carriers and binders for the essential nutritive elements, were conducted before a medium possessing the proper physical qualities was formulated. Cellulose, supplemented with a small quantity of some cereal preparation such as cornstarch, corn meal, or oatmeal, and in some tests gelatin, as a binder, constituted the carrier or foundation for the nutritive elements. While such media sufficed to maintain life and foster a certain degree of growth in a few of the test larvae, in edibility and moisture-retaining ability they were not satisfactory. Furthermore, it was desired to eliminate all cereal matter from the media because of chemical complexities. Agar was one of the materials tested independently as a carrier and temporarily discarded, but its nonnitrogenous character, water-carrying capacity, and ability to remain congealed at incubator-room temperatures again suggested its possible utility. Accordingly, hot liquid agar was mixed with pulverized cotton in an effort to form a more satisfactory carrier. The carrier finally used was made by dissolving shredded agar in hot water and mixing it with cellulose that had been soaked in water. The material was then placed in a water bath and boiled 10 minutes. Known quantities of the dry nutritive elements and fats were then added, and the medium was stirred or beaten vigorously until it was of a homogeneous texture. As the heat treatment might reduce the dietary properties of some of the vitamins, particularly B<sub>1</sub>, the composition was allowed to cool to near room temperature before they were added.

Various combinations of moisture, carbohydrates, proteins, fats, and vitamins were used in the 20 tests made with this carrier (table 1). The quantity of total mineral matter was also varied, but the same compounds in the same proportion (table 2) were used in all except formulas B-6, B-10, and B-14, in which the minerals occurring naturally in the cereal component of the media supplied these elements.

The larvae used in the tests were progeny of field-run moths of the mixed races that now inhabit the Toledo locality; i. e., both single- and multiple-generation borers and hybrids of the two strains. Mature corn borer larvae collected from a single field in the fall were isolated and kept in cold storage at 38°-40° F. until 3 to 4 weeks prior to the start of an experiment. They were then placed in an incubator at 80° under moisture conditions suitable for pupation and moth emergence. Larvae just hatched from eggs laid by the moths were employed for infesting all materials tested.

The tests were conducted in an incubator room in which a constant temperature of 80°±1° F. and a minimum relative humidity of 70 percent were maintained.

TABLE 1.—*Synthetic food formulas tested in the laboratory for rearing larvae of the European corn borer*

Formula	Water	Cellulose	Agar	Proteins			Carbohydrates			Fat	Mineral	Vitamins (International units)				
				Peptone	Casein	Zein	Glucose	Sucrose	Starch			A	B <sub>1</sub>	C	D	E
	Cc.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.					
B-1	90	3.5	3.0	0.02	2.0	1.0	2.0	1.0	0	0.3	0.3	90	150	0	127.5	1
B-2	90	3.0	3.0	.04	2.0	0	2.0	1.0	0	.3	.3	90	150	0	627.5	1
B-3	90	3.5	3.0	0	2.0	0	2.0	1.0	0	.3	.3	900	0	250	225	1
B-4	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	0	0	90	0	0	127.5	0
B-5	75	3.5	2.0	.01	1.5	0	1.5	1.5	0	1.0	.2	0	150	0	0	0
B-6	70	3.0	2.0	.03	2.5	0	2.5	3 13.6	0	1.4	1.3	0	0	0	0	0
B-7	70	3.5	1.0	.02	2.5	0	2.0	1.0	0	1.0	.6	0	150	0	0	0
B-8	85	3.5	3.0	0	2.0	0	2.0	1.0	0	.3	.3	0	150	250	0	1
B-9	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	.3	.3	0	0	0	0	0
B-10	72	3.0	2.0	.02	2.0	0	2.0	3 13.6	0	1.4	1.3	0	0	0	0	0
B-11	85	3.5	3.0	.02	2.0	0	2.0	1.0	0	0	.3	150	0	0	0	0
B-12	85	3.5	3.0	.20	2.0	2.0	2.0	1.0	0	.2	.3	90	150	0	1,627.5	1
B-13	75	2.5	2.5	0	2.0	0	2.0	1.0	0	.3	.3	1,200	150	200	300	1
B-14	75	3.0	2.0	0	2.0	0	2.0	3 13.6	0	1.4	1.3	0	0	0	0	0
B-15	70	2.5	2.0	.02	1.0	0	1.5	1.0	1.0	0	.5	0	150	0	0	0
B-16	70	2.5	2.0	.03	1.0	1.5	2.0	0	0	.3	.3	90	150	0	127.5	0
B-17	70	3.5	2.0	.01	1.5	0	1.5	1.5	1.0	.5	.3	0	0	0	0	0
B-18	85	3.0	3.0	0	0	2.0	2.0	1.0	0	0	.3	0	0	0	0	0
B-19	90	3.0	3.0	0	0	0	2.0	1.0	0	.3	.3	0	0	0	0	0
B-20	85	3.0	3.0	0	0	0	0	0	0	0	0	0	0	0	0	0

<sup>1</sup> Vitamin E was supplied in wheat-germ oil, indicated as fat, concentration unknown.

<sup>2</sup> Includes 2.0 gm. of casein plus 3.2 gm. of cereal proteins.

<sup>3</sup> Total carbohydrates as supplied in cereal component of these media.

<sup>4</sup> Total proteins as supplied in cereal component of these media.

TABLE 2.—*Inorganic mineral salts in synthetic food media tested for rearing larvae of the European corn borer*

Compound	Formula	Proportionate amount
Potassium dihydrogen phosphate.....	$\text{KH}_2\text{PO}_4$ .....	1 gm.
Calcium sulfate.....	$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .....	1 gm.
Ferric chloride.....	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ .....	1 gm.
Magnesium sulfate.....	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	1 gm.
Copper ammonium hydroxide.....	$\text{Cu}(\text{NH}_3)_4(\text{OH})_2^1$ .....	0.13 ml. <sup>1</sup>

<sup>1</sup> A saturated solution of this compound was prepared by bringing together ammonium hydroxide and cuprous oxide. The copper salt was added in excess of ammonium hydroxide.

## PRESENTATION OF RESULTS

The corn borer was reared to maturity on an entirely synthetic food medium and so far as the author knows, this is the first instance of a phytophagous insect being so reared. Although these tests were designed primarily to determine whether the insect could be reared to maturity on a synthetic food medium, rather than to show the specific influences of the various elements utilized, the results do indicate the possibilities of this approach to studies of insect-nutrition requirements and provide general interpretations of the function of some of the more important constituents of the media.

Average percentages of survival at the end of 30 days, pupation at the end of 40 days, and weights of test larvae 20, 30, and 40 days old, reared on 20 samples of synthetic food media, are presented in table 3. The data are arranged to conform with the descending order of weights of 30-day-old larvae.

TABLE 3.—*Larval survival at end of 30 days, average weights of larvae as a result of feeding for 20, 30, and 40 days, and total pupation of surviving corn borer larvae at the close of the 40-day test period*

Formula	Average larval survival at end of 30 days	Average weight per larva at end of—			Total pupation of larvae surviving at end of 40 days
		20 days	30 days	40 days	
	Percent	Milligrams	Milligrams	Milligrams	Percent
B-1 .....	8.1	22.4	81.5	83.8	9.2
B-2 .....	14.0	32.7	72.0	81.3	16.7
B-3 .....	13.0	24.0	69.1	62.6	25.0
B-4 .....	15.0	21.3	65.3	69.1	0
B-5 .....	4.2	15.6	61.4	79.5	20.0
B-6 .....	8.0	36.7	58.5	74.3	23.5
B-7 .....	11.8	18.2	56.4	61.5	0
B-8 .....	17.2	22.6	54.0	64.0	0
B-9 .....	36.0	31.9	47.6	-----	30.0
B-10.....	3.3	36.0	46.4	88.7	0
B-11.....	20.0	21.7	42.4	55.0	0
B-12.....	11.3	18.0	38.2	-----	0
B-13.....	6.0	18.2	36.5	61.5	25.0
B-14.....	7.2	28.0	34.7	38.0	0
B-15.....	11.0	15.5	33.3	42.8	0
B-16.....	21.0	6.9	23.0	38.6	12.5
B-17.....	.4	4.0	22.2	89.0	0
B-18.....	4.5	4.6	17.8	23.5	0
B-19.....	1.5	4.1	15.5	18.0	0
B-20.....	0	-----	-----	-----	0



While it is indicated that nutrition has some influence on the number of generations produced, all the differences in percentages of pupation between lots of test larvae herein reported are not due solely to variation in nutrition. The studies show that the growth rate and the inherent size limitation of the insect are closely related to the character that influences the number of generations produced. Generally, the multiple-generation larvae never attained so great a weight as the single-generation larvae, which grow more slowly. Of those larvae that survived, as many as 30 percent pupated in one test and 20 percent or more in four other tests.

### DISCUSSION OF RESULTS

In general, the most satisfactory synthetic media for rearing larvae of the European corn borer to a physiological maturity that will permit normal pupation were formulated approximately as follows: Cellulose 3.5 gm., agar 3 gm., casein 2 gm., glucose 2 gm., sucrose 1 gm., total mineral salts 0.35 gm., fat 0.3 to 1.0 gm., and water 85 cc.

The pH of such media averaged about 4.5 immediately after they were mixed, but it was somewhat lower after they had stood in the incubator room 24 hours or more. Media in which zein was employed as the sole source of proteins underwent greater changes in pH (lower readings) than did media in which casein was used. Lower pH readings were usually associated with higher larval mortality and relatively low weights of those larvae that did survive.

### EFFECT OF NUTRIENTS ON LARVAL DEVELOPMENT

As many as 36 percent of corn borer larvae nourished entirely on a synthetic food medium survived to maturity and attained near-normal weights during a 30- to 40-day test period. Larval survivals of about 20 percent are obtained on corn under average field conditions and from 30 to 60 percent on favorable food under laboratory conditions.

The development of single-generation larvae confined to synthetic foods was retarded as compared with that of larvae reared at the same time and under the same environmental conditions on fresh green plant food. Likewise, the time prior to pupation of multiple-generation larvae when reared on synthetic foods was 3 or more days longer than that generally required when reared on green plant tissue.

Low survivals resulting from formulas B-10 and B-17 are probably due to factors other than nutrition, since the few surviving larvae attained satisfactory weights. The nonsurvival on B-20 as early as the tenth day after infestation was due to lack of nutritive substance, since the cellulose-agar base was unsupplemented for this check test.

### PROTEINS

Low survivals, low weights, and nonpupation of surviving larvae on B-18 and B-19 are attributed to incompatibility of zein with other components of the media and to the lysine and other amino acid deficiencies peculiar to this protein. Superiority of casein to zein is indicated. It is known that casein contains more of the amino acids essential to the nutrition of higher animals than does zein, and it appears highly probable that other proteins present in the corn

plant and grain, which supply such amino acids as lysine, tryptophane, and histidine, are essential to the nutrition of the corn borer. Although casein is the best protein tested to date and serves as a rich source of lysine and most of the other dietary-essential amino acids, it is deficient in cystine, necessary for supporting growth in rats,<sup>5</sup> and this deficiency may have been largely responsible for the dwarfness among the borers supplied solely with this source of protein, as compared with those reared on plant tissue. Peptone employed as a supplement to either zein or casein appeared to stimulate feeding and subsequent growth of the test larvae. The legume proteins contain relatively large amounts of lysine, tryptophane, and histidine, in marked contrast to those of corn, a fact which probably materially contributes to the nutritional adaptiveness of beans and peas for successfully rearing the corn borer in the laboratory.

#### CARBOHYDRATES

A 2-to-1 ratio of glucose to sucrose seems to meet the carbohydrate requirements of the borer. Absence of biological response to the inclusion of cornstarch in the food media is in full accord with the results of enzyme tests reported by Bottger,<sup>6</sup> in which the borer's inability to digest cornstarch was indicated by the negative reaction of the tests for amylase, the starch-splitting enzyme.

#### FATS

Fat requirements of the borer cannot be established on the basis of results from these tests, but nearly all the more satisfactory media contained some fat either as a carrier for vitamins or as an independent constituent. Fats were supplied in the form of corn oil, wheat-germ oil, and halibut-liver oil.

#### MINERALS

Previous tests indicated that inclusion of several of the minerals found in corn greatly improves the nutritive qualities of synthetic media for the borer. Mineral salts were therefore included in all media tested. The buffering effect of certain minerals was of value in neutralizing the acidity attending some other components of the media. The inclusion of copper ammonium hydroxide also appeared to improve the physical properties of the media, thereby aiding the borer to spin its silken cocoon, or feeding web, which seems to be essential for normal feeding. Sodium chloride was included among the minerals employed in some earlier tests, but omitted in the series herein reported because it did not appear to improve the nutritive qualities of the media and because its concentration in the corn borer's natural host plant is believed to be relatively low. Latshaw and Miller<sup>7</sup> do not mention sodium in their report on the elemental composition of the corn plant.

<sup>5</sup> SHERMAN, HENRY C. *CHEMISTRY OF FOOD AND NUTRITION*. Ed. 5, completely rewritten, 640 pp., illus. New York, 1937. See pp. 71-74.

<sup>6</sup> See footnote 3.

<sup>7</sup> See footnote 4.

## VITAMINS

There is some indication that vitamins A, B<sub>1</sub> (thiamin chloride), and possibly E, improved certain media. However, when protein was supplied in the form of casein or peptone and constituted 3 percent or more of the total constituents of the media, the vitamins did not appear to be essential for growth and development of the corn borer. No particular biological response to either vitamin C or vitamin D was evident.

## EFFECT OF SYNTHETIC FOOD ON THE METAMORPHOSIS AND REPRODUCTION OF THE CORN BORER

The length of the pupal stage of insects reared on the more favorable synthetic media was the same as for those reared on natural foods, and the moths emerged normally from such pupae. Females reared on the synthetic media laid infertile eggs either when unmated or when given opportunity to mate with males reared on synthetic food. However, poor synchronization of emergence of the limited numbers of both sexes is believed to have prevented coitus. When a newly emerged male reared on a synthetic medium was mated with a virgin female reared on plant tissue, normal mating and deposition of fertile eggs resulted. Although attempts to mate females reared on synthetic media, which attained subnormal size in these experiments, with plant-reared normal-sized males were unsuccessful, it seems probable that such adults possessed the ability to reproduce under favorable circumstances. As female moths are normally larger than males, the reversal in size differences of the sexes in this attempted cross may have made the sexual union of these individuals physically impossible.

## SUMMARY

Information accumulated from various nutrition studies of the European corn borer (*Pyrausta nubilalis* (Hbn.)) was used in conducting a series of tests for the purpose of rearing larvae to maturity on a synthetic food medium.

Twenty food media were formulated to approximate the chemical composition of green corn tissue. The media were immediately placed in glass rearing receptacles and infested with newly hatched larvae. The tests were conducted in a room-size incubator where a constant temperature of  $80^{\circ} \pm 1^{\circ}$  F. and a minimum relative humidity of 70 percent were maintained.

Since the test larvae were parented by field-run stock of the mixed races of the European corn borer now occurring in the vicinity of Toledo, Ohio, both single- and multiple-generation strains resulted. The expression of the single- or multiple-generation characters is probably influenced by nutrition, but all the differences in pupation between tests should not be attributed to nutritive effects.

As many as 36 percent of the corn borer larvae confined to synthetic food survived to maturity; in one test as many as 30 percent, and in four other tests 20 percent or more larvae pupated.

Superiority of casein to zein as a source of protein was indicated. Peptone employed as a supplement to either casein or zein appeared to stimulate both feeding and growth of larvae.

A 2-to-1 ratio of glucose to sucrose appeared to satisfy the carbohydrate requirement of the corn borer.

Fat requirements of the corn borer cannot be established on the basis of these tests, but most of the more satisfactory media contained some fat.

Inorganic mineral salts were included in all media. Previous tests have indicated that certain mineral salts are nutritionally beneficial to corn borer larvae and also tend to buffer the media against excessive acidity.

There was some indication that vitamins A, B<sub>1</sub> (thiamin chloride), and E are of nutritional value to the corn borer, particularly in the absence of a sufficiently high percentage of casein in the food medium.

The duration of the pupal stage was normal, and moths of both sexes emerged normally. Poor synchronization of emergence of the limited numbers of male and female moths is believed to have prevented normal mating and the deposition of fertile eggs.



# JOURNAL OF AGRICULTURAL RESEARCH

## CONTENTS

	Page
Inheritance and Interrelationship of Components of Quality, Cold Resistance, and Morphological Characters in Wheat Hybrids (Key No. Ind.-31) . . . . .	501
W. W. WORZELLA	
The Response of "Ceased" Reactors in Bang's Disease to Recurrence (Key No. Wis.-134) . . . . .	523
B. A. BEACH, M. R. IRWIN, AND L. C. FERGUSON	



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## INHERITANCE AND INTERRELATIONSHIP OF COMPONENTS OF QUALITY, COLD RESISTANCE, AND MORPHOLOGICAL CHARACTERS IN WHEAT HYBRIDS<sup>1</sup>

By W. W. WORZELLA

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### INTRODUCTION

In the soft red winter wheat region one of the main problems in the breeding program is that of developing new varieties that not only possess suitable quality but also are winter hardy. In order to "build up" or synthesize such new varieties most directly it is essential to obtain information on the mode of inheritance of quality and winter hardiness, and to determine the extent of correlation or linkage, if any, among these characters.

This paper reports a study of the inheritance and interrelationship of components of quality, cold resistance, and morphological characters in wheat (*Triticum aestivum* L.) hybrids. It was carried out on 600 hybrids and the parent stocks originating from a triangular cross of American Banner, Trumbull, and Michikof, grown at Lafayette, Ind., in 1937-38 and repeated in 1939-40.

### REVIEW OF LITERATURE

Several reports have been published on the inheritance of components of quality, cold resistance, and morphological characters in wheat, but few data are available on the interrelationships of these characters, especially with hybrid populations selected at random. Since 11 different characters are taken up in this report, only the more pertinent papers will be reviewed.

Workers have used loaf volume, fermentation time, extensimeter, and swelling number as indices of gluten strength. Meneret (32)<sup>2</sup> and Rosenstiel (36) found two pairs of factors, while Worzella (43) found three pairs of factors to govern the mode of inheritance of this character. Data obtained by Alabouvette (5), Ausemus et al. (8), Cutler and Worzella (20), Hayes et al. (27), Saunders (37, 38), and Worzella and Cutler (46) indicate that the inheritance of gluten strength is governed by multiple factors.

Aamodt and Torrie (2), Biffen (9, 10), Bryan and Pressley (12, 13), and Howard and Howard (28) reported that a single genetic factor conditioned the inheritance of kernel texture. Two factors for kernel texture were reported by Aamodt and Torrie (2), and Freeman (22), while polymeric factors were found by Aamodt et al. (3), Clark et al. (16), Harrington (24), Hayes (25), and Hayes et al. (27).

<sup>1</sup>Received for publication Jan. 24, 1942. Journal Paper No. 2 of the Purdue University Agricultural Experiment Station.

<sup>2</sup>Italic number in parentheses refer to Literature Cited, p. 520.

Working with a series of varieties and hybrid strains, Whiteside (41), Whiteside et al. (42), and Worzella and Cutler (45, 46) showed that the carotenoid pigment content in wheat is genetic in nature. Clark and Smith (19) and Markley (31) reported that polymeric factors govern the inheritance of carotenoid content in durum wheats.

Multiple factor inheritance for crude protein content was indicated by Aamodt and Torrie (2), Ausemus et al. (8), Clark (15), Clark et al. (16), Clark and Hooker (17), Clark and Quisenberry (18), Clark and Smith (19), Hayes et al. (27), and Zinn (49).

The mode of inheritance of kernel weight was reported by Jasnowski (29, 30) to be governed by three factor pairs.

Åkerman (4), Hayes and Aamodt (26), Nilsson-Ehle (33), Quisenberry (34), Quisenberry and Clark (35), and Worzella (44), working with different wheat crosses, concluded that cold resistance is a heritable character and is controlled by many genetic factors.

Aamodt and Torrie (2), Ausemus et al. (8), Clark (15), Clark and Smith (19), Goulden et al. (23), Hayes et al. (27), Waldron and Mangels (40), Whiteside et al. (42), and Zinn (49) studied the interrelationship of factors for quality, such as protein content, loaf volume, texture, color, test weight, etc., on a series of varieties and selected hybrid strains. Data obtained by these workers show a general tendency for an association between protein content and loaf volume, carotenoid content and crumb score, and kernel texture and protein content; however, many of the correlation coefficients were low in magnitude while others were not significant. Intercharacter correlations between other components of quality usually were not significant or consistent under different conditions.

Many investigators have reported that the mode of inheritance of glume and kernel color is governed by one, two, or three pairs of genetic factors. Ausemus (7) and Churchward (14) showed that one factor pair governs the inheritance of coleoptile color, while Quisenberry (34) found two factor pairs. The inheritance of straw color was reported by Torrie (39) to be monogenic. Churchward (14) found a strong correlation between straw and coleoptile color.

#### MATERIALS AND METHODS

The experiments here reported were conducted with  $F_3$ ,  $F_4$ , and parent rows grown under field conditions during 1937-38 and repeated in 1939-40. The three common wheat varieties American Banner, Trumbull, and Michikof were used as parents in this study. Table 1 shows the contrasting characters of the three varieties.

Reciprocal crosses between pure lines of American Banner, Trumbull, and Michikof were made in the greenhouse in 1935 and  $F_1$  plants were grown during 1935-36. The  $F_2$  generation was grown in the field at the soils and crops farm at Lafayette, Ind., during 1936-37. The kernels were spaced at 4-inch intervals in 18-foot rows 1 foot apart.



TABLE 1.—Contrasting characters of the parent varieties American Banner, Trumbull, and Michikof

Contrasting characters	Variety			Difference necessary for significance between 2 means (Odds 19:1)
	American Banner	Trumbull	Michikof	
Gluten strength.....minutes..	18.8	31.4	195.6	16.42
Particle-size index.....percent..	16.5	17.8	8.8	.87
Carotenoid content.....p. p. m..	1.87	1.95	2.09	.08
Protein content.....percent.....	9.1	9.6	10.6	.30
1,000-kernel weight.....grams....	36.0	35.2	31.9	1.00
Test weight.....pounds.....	57.5	59.2	60.2	.67
Cold resistance.....percent.....	20.6	24.2	50.9	1.09
Glume color.....	Brown	White	White	-----
Kernel color.....	White	Red	Red	-----
Coleoptile color.....	Green	Purple	Green	-----
Straw color.....	White	Purple	White	-----

<sup>1</sup> Percentage below the mean (Worzella, 43, pp. 707-708).

A random selection of the  $F_2$  populations was made as a basis for continuing the study in the  $F_3$  generation. Two hundred  $F_2$  plants were selected from each of the three crosses. In the fall of 1937, 50 grains from each  $F_2$  plant were used to seed an  $F_3$  progeny row by spacing the kernels at 3-inch intervals in 12-foot rows 1 foot apart. Check rows of the parent varieties, sown in the same manner, were alternated every tenth row. Following a favorable growing season, from 35 to 48 plants per row were available for harvest in 1938. Representative wheat samples for each row were obtained by harvesting 3 heads from each plant and threshing them in bulk. In addition, 1 head from each  $F_3$  hybrid plant was selected for  $F_4$  progeny tests. The 666 composite samples, representing the parents and  $F_3$  progenies of the 1938 crop, were analyzed for several components of quality and cold resistance.

In the fall of 1939, 200 grains from each  $F_3$  family were planted in 6-foot rows 1 foot apart. Check rows of the parent varieties, sown in the same manner, were alternated every tenth row. In addition, several  $F_3$  families that showed wide differences in respect to quality were further progeny tested in 4-foot head rows in the  $F_4$  generation. Each row was harvested separately and threshed in bulk. Representative samples from each row were used to conduct the quality studies of the 1940 crop.

Gluten strength was determined by the wheat-meal fermentation-time test as described by Cutler and Worzella (21). Granulation or the particle-size index of wheat meal was determined according to the method developed by Worzella and Cutler (47). The method reported by Binnigton and Geddes (11) with some minor modifications was used for determining the carotenoid content of wheat. A 10-gm. sample of finely ground wheat meal was placed in a 4-ounce bottle containing 50 cc. of water-saturated butanol and allowed to stand for 16 hours with occasional shaking. Clarification was effected by filtration through No. 1 Whatman paper. The yellow pigment content of the extract was determined in a 2-cm. cell, using a KWSZ photometer. Pure beta-carotene was used to prepare a series of known standards. By means of a conversion table the percentage transmittancy readings were expressed as parts of carotene per million parts of wheat meal. Crude-protein determinations were made

according to the method outlined in Cereal Laboratory Methods (6). Protein data are reported on a 13.5 percent moisture basis. The relative test weights of the small samples of wheat were determined by the method developed by Aamodt and Torrie (1). Cold-resistance studies were conducted under artificial freezing tests according to the method described by Worzella and Cutler (48).

### EXPERIMENTAL RESULTS

Genetic analyses and experimental data on gluten strength, granulation, carotenoid pigment content, crude protein, kernel weight, test weight, cold resistance, and several morphological characters are presented in the order named. Following these, the relationships between the characters are reported.

#### INHERITANCE OF CHARACTERS

##### GLUTEN STRENGTH

To study the inheritance of gluten strength, fermentation-time tests were made on the grain obtained from  $F_3$  and  $F_4$  hybrids and parents, originating from a triangular cross of American Banner, Trumbull, and Michikof, grown at Lafayette, Ind., in 1937-38 and

TABLE 2.—Frequency distribution of fermentation time for gluten strength of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Generation	Year	Lines in class center for fermentation time in number of minutes stated <sup>1</sup>										Lines	Mean	Coefficient of variability <sup>2</sup>
			18.0	24.4	33.1	44.9	60.8	82.4	111.8	151.4	205.2				
American Banner .....	P <sub>1</sub>	1938	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Min.		
Do .....	P <sub>1</sub>	1940	20	2									22	18.5	8.4
Trumbull .....	P <sub>1</sub>	1938	18	4									22	19.1	8.6
Do .....	P <sub>1</sub>	1940		1	21								22	32.7	8.9
Michikof .....	P <sub>1</sub>	1938		7	15								22	30.1	13.9
Do .....	P <sub>1</sub>	1940								3	19		22	196.9	10.5
AB × T .....	F <sub>3</sub>	1938								4	18		22	194.2	11.3
Do .....	F <sub>3</sub>	1940	54	120	25	1							200	23.5	17.4
AB × M .....	F <sub>3</sub>	1938	51	111	37	1							200	24.0	18.6
Do .....	F <sub>3</sub>	1940	1	2	37	75	54	14	9	6	2		200	52.2	32.8
T × M .....	F <sub>3</sub>	1938		3	31	77	46	17	15	10	1		200	54.1	33.0
Do .....	F <sub>3</sub>	1940			5	36	64	42	29	21	3		200	74.0	33.8
Pedigree No.:	F <sub>4</sub>	1940			11	27	40	46	35	33	8		200	82.2	37.7
76 .....	F <sub>4</sub>	1940	24	2									26	17.2	8.2
635 .....	F <sub>4</sub>	1940	7	13	1								21	22.4	15.7
299 .....	F <sub>4</sub>	1940			1	4	14	5	3	1			28	66.2	27.9
650 .....	F <sub>4</sub>	1940				1	3	3	6	4	1		18	101.0	33.3
603 .....	F <sub>4</sub>	1940							1	11	4		16	166.5	13.6
266 .....	F <sub>4</sub>	1940								1	21		22	202.3	8.8

<sup>1</sup> A logarithm of 0.132 was used in calculating class intervals (Worzella, 43, p. 707).

<sup>2</sup> Percentage below the mean (Worzella, 43, pp. 707-708).

1939-40. The data obtained were arranged in frequency distributions and are shown in table 2 and in figure 1.

The results show that the three parent varieties are widely different in gluten strength. The average time of disintegration for dough balls made from American Banner, Trumbull, and Michikof, was about 19, 31, and 195 minutes, respectively.

The gluten quality of the  $F_2$  families, in each cross, varied all the way from that of the weaker to that of the stronger parent. The frequency distributions of fermentation time for the hybrids from the

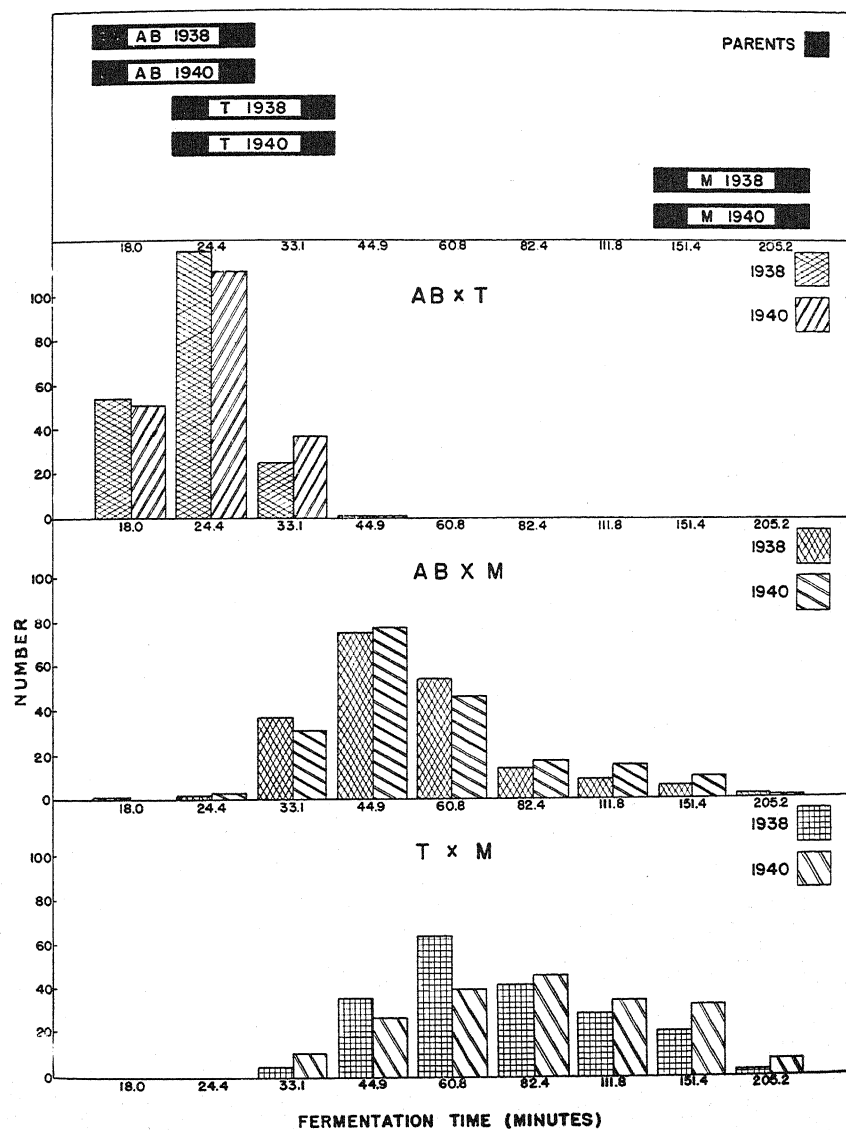


FIGURE 1.—Range in fermentation time for gluten strength of the parent varieties American Banner, Trumbull, and Michikof, and  $F_2$  distribution of fermentation time for gluten strength of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses. Crops of 1938 and 1940.

American Banner  $\times$  Michikof and Trumbull  $\times$  Michikof crosses, approach normal curves. There is, however, a preponderance of hybrids toward the short time side of the frequency distribution

which may be due to the effect of environmental conditions favoring the development of weak gluten wheats.  $F_4$  families just as weak as American Banner and just as strong as Michikof in gluten strength were recovered. Some of the  $F_4$  lines (76 and 266) bred comparatively true, the range for time being no greater than for the parents, while others varied as much as the  $F_3$  generation ( $F_2$  distribution).

The sharp rise in the variability between the parents and later generations, the recombination of families possessing various degrees of gluten strength, and the reappearance of parental types, indicate clearly the existence of segregation of genetic factors. Since about one-fourth of the families in the American Banner  $\times$  Trumbull cross possess approximately the same gluten quality as that found in American Banner, it would appear that 1 major factor pair governs the mode of inheritance of gluten strength in this cross. Based on the number of parental recombinations, when only 200 families were studied, the data indicate that about 4 factor pairs are involved in the inheritance of gluten strength in the American Banner  $\times$  Michikof cross, while about 3 factor pairs may explain the genetic difference between the Trumbull and Michikof varieties. The writer is not unmindful of the fact that such a genetic analysis may not always hold true, since environment greatly influences the expression of the gluten quality character.

#### GRANULATION

Meal granulation, or degree of particle fineness, is an important component of wheat quality. In studying its mode of inheritance, granulation tests were conducted on finely ground wheat meal of  $F_3$  and  $F_4$  hybrids originating from three crosses. The data, expressed in percentage and designated as particle-size index, are given in table 3 and figure 2.

TABLE 3.—Frequency distribution of particle-size index of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Gen- era- tion	Year	Lines in class center for particle-size index of wheat meal in percentage stated												Lines	Mean	Standard deviation
			23.4	21.8	20.2	18.6	17.0	15.4	13.8	12.2	10.6	9.0	7.4				
			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.				
American Banner	P <sub>1</sub>	1938	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Pct.			
Do	P <sub>1</sub>	1940			5	6	4	10	7	1			22	15.1	1.35		
Trumbull	P <sub>1</sub>	1938				7	8	3					22	17.9	1.62		
Do	P <sub>1</sub>	1938				7	9	5	1				22	17.0	1.24		
Do	P <sub>1</sub>	1940	1	2	2	9	8						22	18.7	1.88		
Michikof	P <sub>1</sub>	1938									1	9	12	22	8.3		
Do	P <sub>1</sub>	1940								2	4	14	2	22	9.4		
AB $\times$ T	F <sub>3</sub>	1938			9	52	88	45	3	3				200	17.1		
Do	F <sub>4</sub>	1940	13	39	68	62	16	2						200	19.9		
AB $\times$ M	F <sub>3</sub>	1938			2	6	18	19	29	43	32	31	20	200	12.2		
Do	F <sub>4</sub>	1940				4	15	18	36	47	42	30	8	200	12.3		
T $\times$ M	F <sub>3</sub>	1938			1	5	19	22	29	43	34	31	16	200	12.3		
Do	F <sub>4</sub>	1940			4	10	15	34	47	35	31	20	4	200	13.3		
Pedigree No.:																	
299	F <sub>4</sub>	1940		1	1	4	10	9	2	1				28	16.6		
216	F <sub>4</sub>	1940				3	16	10						29	16.6		
339	F <sub>4</sub>	1940				2	3	4	6	4	3	3	1	26	13.4		
635	F <sub>4</sub>	1940						1	2	3	8	5	2	21	10.7		
322	F <sub>4</sub>	1940									3	5	22	30	8.0		



A comparison of the results of the two seasons shows that the wheat of the 1940 crop produced a finer meal or flour than that harvested in 1938. The average particle-size index of 16.5 and 17.8 for American

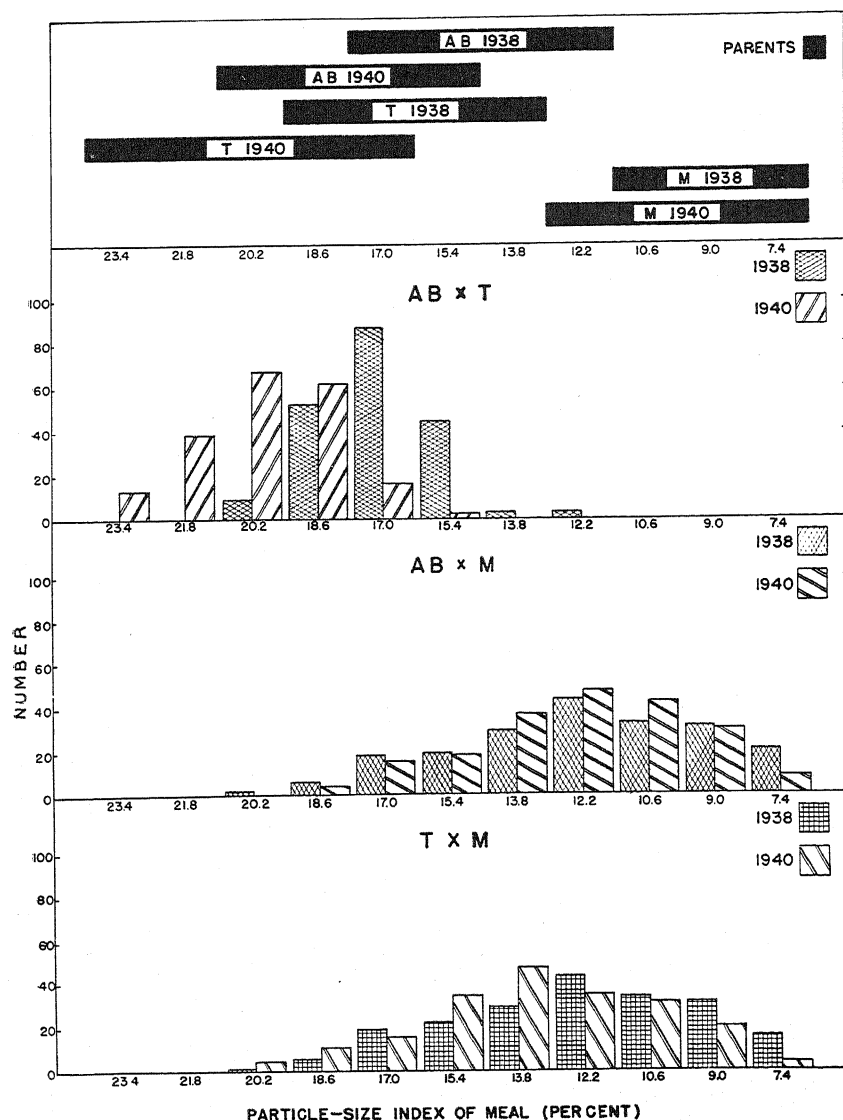


FIGURE 2.—Range in particle-size index of the parent varieties American Banner, Trumbull, and Michikof, and  $F_2$  distribution of particle-size index of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses. Crops of 1938 and 1940.

Banner and Trumbull, respectively, indicates that these varieties grind into a relatively fine meal, whereas the variety Michikof, with an index of 8.8, produces a coarse meal.

The hybrids, considered as a whole, are intermediate to the parents in meal granulation with  $F_3$  families varying in degree of particle fineness from the finer to the coarser grinding parent. The frequency distributions of particle-size index, for each of the 3 hybrid populations, suggest a normal curve.  $F_4$  lines (216 and 322) were selected that varied widely in meal granulation and that bred comparatively true. The results show, therefore, that meal granulation is inherited in the same way as other quantitative characters; however, since parental recombinations reappeared many times in populations of 200, it appears that only a few genetic factors govern the mode of inheritance of meal granulation in the varieties studied.

#### CAROTENOID PIGMENT CONTENT

The amount of carotenoid pigments in wheat usually reflects color, an important characteristic of white flour. Samples of wheat, representing hybrid lines and parent rows, were analyzed for the amount of carotenoid pigments in order to study the mode of inheritance of this character. The data are shown in table 4 and figure 3.

TABLE 4.—Frequency distribution of carotenoid pigments of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Generation	Year	Lines in class center for carotene in parts per million stated												Lines	Mean	Standard deviation
			1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5			
			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.			
American Banner	P <sub>1</sub>	1938	---	6	11	5	---	---	---	---	---	---	---	No.	22	p. p. m.	0.15
Do	P <sub>1</sub>	1940	---	8	13	1	---	---	---	---	---	---	---	22	1.90		
Trumbull	P <sub>1</sub>	1938	---	2	13	7	---	---	---	---	---	---	---	22	1.84	.11	
Do	P <sub>1</sub>	1940	---	2	10	10	---	---	---	---	---	---	---	22	1.93	.11	
Michikof	P <sub>1</sub>	1938	---	---	8	10	4	---	---	---	---	---	---	22	1.97	.14	
Do	P <sub>1</sub>	1940	---	---	3	14	5	---	---	---	---	---	---	22	2.06	.13	
AB × T	F <sub>3</sub>	1938	28	78	73	16	5	---	---	---	---	---	---	200	2.12	.12	
Do	F <sub>4</sub>	1940	3	47	87	50	11	2	---	---	---	---	---	200	1.80	.19	
AB × M	F <sub>3</sub>	1938	2	15	26	34	69	40	10	2	2	---	---	200	1.93	.18	
Do	F <sub>4</sub>	1940	1	21	38	51	53	26	9	1	---	---	---	200	2.04	.29	
T × M	F <sub>3</sub>	1938	7	33	35	52	36	25	10	1	1	---	---	200	1.95	.27	
Do	F <sub>4</sub>	1940	1	19	25	37	41	39	24	10	4	---	---	200	1.90	.32	
Pedigree No.:															2.09	.35	
289	F <sub>4</sub>	1940	3	24	3	---	---	---	---	---	---	---	---	30	1.50	.09	
277	F <sub>4</sub>	1940	---	6	4	9	6	2	1	---	---	---	---	28	1.88	.27	
228	F <sub>4</sub>	1940	---	1	1	5	11	6	---	---	---	---	---	24	2.27	.20	
299	F <sub>4</sub>	1940	---	1	2	10	6	3	4	2	---	---	---	28	2.30	.31	
380	F <sub>4</sub>	1940	---	---	---	---	---	---	4	7	7	6	3	27	3.08	.25	

The actual values for total carotenoid content in wheat shown in table 4 and figure 3 are somewhat lower than usually reported in the literature. There is disagreement among investigators as to the material best suited for the preparation of standards in the calibration of photoelectric instruments. In this study pure beta-carotene, which is not completely soluble in water-saturated butanol, was used.

The results show that the parental varieties do not differ greatly in carotenoid content; however, the variety, Michikof, is significantly

higher in the amount of yellow pigments than the varieties American Banner or Trumbull. In each cross, the  $F_2$  distributions exceed the

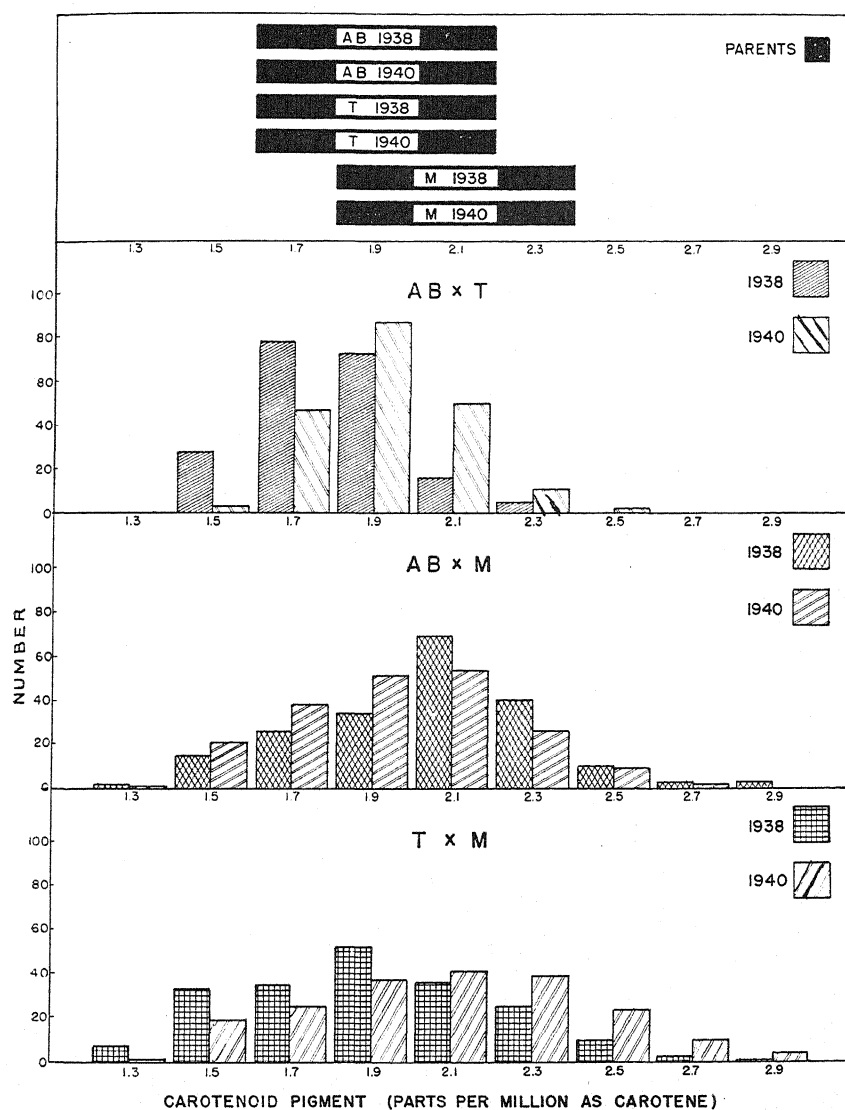


FIGURE 3.—Range in carotenoid pigment content of the parent varieties American Banner, Trumbull, and Michikof, and  $F_2$  distribution of carotenoid pigment content of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses. Crops of 1938 and 1940.

range of the parent varieties, indicating transgressive segregation for carotenoid content.  $F_3$  families as low as 1.3 parts per million and as

high as 2.9 parts per million in carotene were recombined. The frequency distributions of carotene for the hybrid populations approach normal curves.  $F_4$  lines were selected that varied from an average of 1.50 parts per million to 3.08 parts per million in carotene.

The sharp rise in variability between the parents and  $F_3$  generation, and the recombination of lines beyond the range found in the parents (transgressive inheritance), indicates that the mode of inheritance of carotenoid pigment content in wheat is governed by several genetic factors. The data show that although the varieties used in this study varied little in carotenoid content, they are genotypically different and possess different pairs of genetic factors for this character.

#### CRUDE PROTEIN

Crude-protein determinations were made on several hundred samples of wheat, representing hybrids and parent rows, in order to study the manner of inheritance of this character. The data obtained are presented in table 5 and figure 4.

A study of the inheritance of crude-protein content is difficult to make since this character is greatly influenced by environmental conditions. In the 1938 crop, the protein content of the 22 samples of American Banner wheat, all grown within an area of one-fourth acre, varied from 8.5 to 10.0 percent, and for the same year the samples of the variety Trumbull varied from 8.5 to 11.5 percent in protein. Also, the wheat harvested during the 1938 season contained a higher percentage of protein than that of the 1940 crop.

On the basis of the 2 years' data the average protein content of American Banner, Trumbull, and Michikof is 9.1, 9.6, and 10.6 percent, respectively. The differences between these values are statistically significant, indicating that the varieties used in this study are genetically different in crude-protein content. The hybrids, considered as a whole, are intermediate to the parents, with lines varying in percentage protein from the lower to the higher parent.  $F_4$  families that varied significantly in protein content were selected. The data indicate that the mode of inheritance of crude-protein content is conditioned by multiple factors.

TABLE 5.—Frequency distribution of percentage of protein of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Generation	Year	Lines in class center for protein content in percentage stated										Lines	Mean	Standard deviation
			7.8	8.3	8.8	9.3	9.8	10.3	10.8	11.3	11.8	12.3			
			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.			
American Banner.....	$P_1$	1938	---	---	7	9	6	---	---	---	---	---	22	9.3	0.32
Do.....	$P_1$	1940	---	6	8	5	2	1	---	---	---	---	22	8.9	.56
Trumbull.....	$P_1$	1938	---	1	6	9	4	---	1	1	---	---	22	9.9	.56
Do.....	$P_1$	1940	---	3	11	7	1	---	---	---	---	---	22	9.4	.39
Michikof.....	$P_1$	1938	---	---	---	---	2	9	5	3	3	---	22	10.8	.57
Do.....	$P_1$	1940	---	---	---	1	4	11	5	1	---	---	22	10.3	.45
AB $\times$ T.....	$F_3$	1938	---	3	43	100	44	8	---	---	---	---	200	9.3	.43
Do.....	$F_3$	1940	1	14	56	75	48	5	1	---	---	---	200	9.2	.49
AB $\times$ M.....	$F_3$	1938	---	---	4	23	60	51	36	20	3	3	200	10.2	.69
Do.....	$F_3$	1940	1	8	51	90	39	11	---	---	---	---	200	9.3	.47
T $\times$ M.....	$F_3$	1938	---	---	2	10	52	65	54	13	4	---	200	10.3	.56
Do.....	$F_3$	1940	---	---	5	40	86	51	17	1	---	---	200	9.9	.47
Pedigree No.:															
20.....	$F_4$	1940	---	---	11	14	5	---	---	---	---	---	30	9.2	.36
299.....	$F_4$	1940	---	---	---	1	8	12	4	2	1	---	28	10.3	.55
228.....	$F_4$	1940	---	---	---	---	---	---	9	6	5	4	24	11.4	.56



## KERNEL WEIGHT

The 1,000-kernel weight of wheat, representing  $F_3$  families and parent rows, was determined when grown under similar environmental

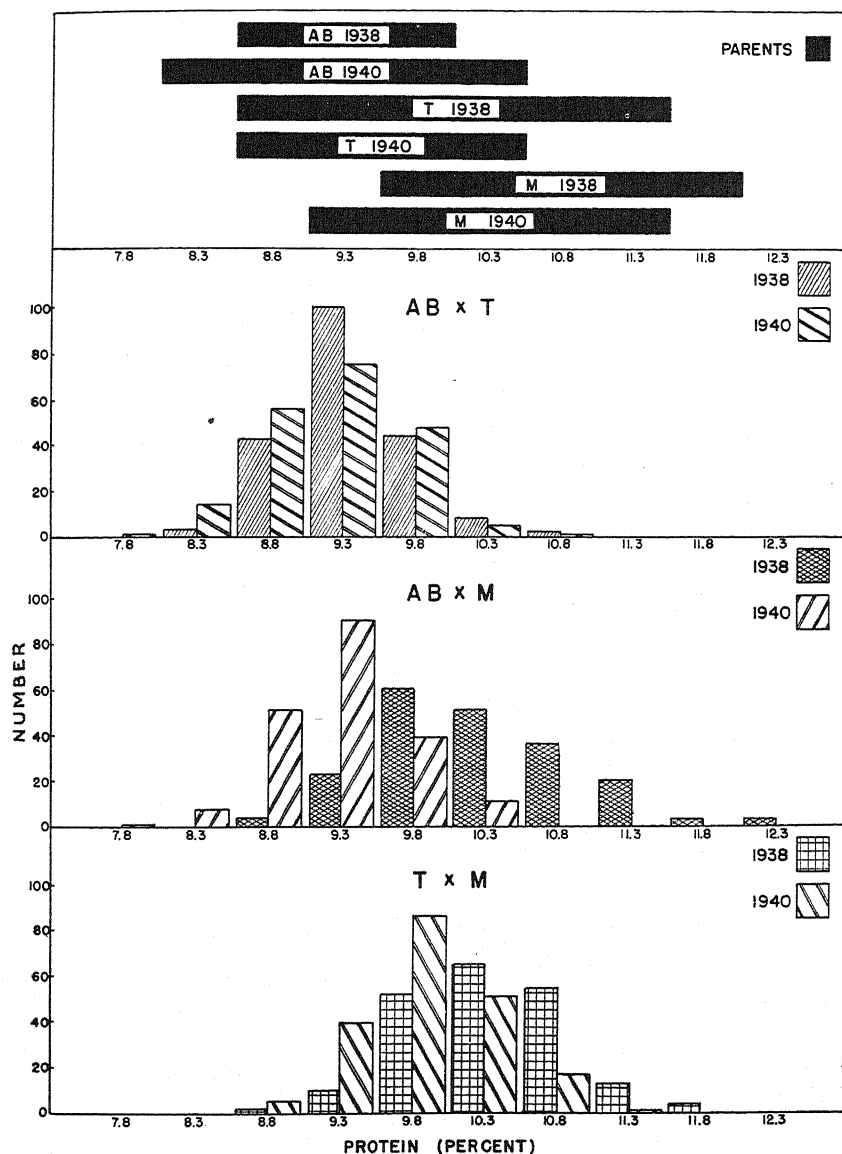


FIGURE 4.—Range in crude-protein content of the parent varieties American Banner, Trumbull, and Michikof, and  $F_2$  distribution of crude-protein content of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses. Crops of 1938 and 1940.

conditions. The data for these and also for several selected  $F_4$  families are presented in table 6.

TABLE 6.—Frequency distribution of weight of 1,000 kernels of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Generation	Year	Lines in class center for 1,000-kernel weight in number of grams stated										Lines	Mean	Standard deviation
			25	27	29	31	33	35	37	39	41	43			
			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.			
American Banner	P <sub>1</sub>	1938	---	---	1	2	---	---	---	---	---	---	22	34.6	2.37
Do.	P <sub>1</sub>	1940	---	---	---	---	---	3	4	1	---	---	22	37.5	1.54
Trumbull	P <sub>1</sub>	1938	---	---	---	---	5	11	6	---	---	---	22	35.1	1.28
Do.	P <sub>1</sub>	1940	---	---	---	1	1	12	8	---	---	---	22	35.4	1.50
Michikof	P <sub>1</sub>	1938	---	---	1	11	9	1	---	---	---	---	22	31.6	1.27
Do.	P <sub>1</sub>	1940	---	---	1	8	12	1	---	---	---	---	22	32.2	1.34
AB $\times$ T	F <sub>3</sub>	1938	---	4	9	21	36	56	42	25	7	---	200	36.9	3.06
Do.	F <sub>3</sub>	1940	---	---	2	18	52	82	39	7	---	---	200	36.6	2.00
AB $\times$ M.	F <sub>3</sub>	1938	---	---	4	37	47	48	16	1	---	---	200	34.5	2.60
Do.	F <sub>3</sub>	1940	---	---	5	31	53	64	36	10	1	---	200	34.3	2.37
T $\times$ M.	F <sub>3</sub>	1938	---	---	3	15	51	82	37	11	---	1	200	35.7	2.15
Do.	F <sub>3</sub>	1940	---	---	7	46	78	49	19	1	---	---	200	34.3	2.02
Pedigree No.:															
299	F <sub>4</sub>	1940	5	8	9	6	---	---	---	---	---	---	28	28.2	2.04
216	F <sub>4</sub>	1940	---	---	1	14	11	3	---	---	---	---	29	32.1	1.52
76	F <sub>4</sub>	1940	---	---	---	3	11	10	2	---	---	---	26	33.8	1.62
5	F <sub>4</sub>	1940	---	---	---	---	1	10	13	3	1	---	28	36.5	1.82

The kernel weight of wheat is greatly influenced by environmental conditions as indicated by the wide range found in the parent varieties. The results show that the varieties American Banner and Trumbull possess large kernels while the kernels of the variety Michikof are considerably smaller. The hybrid populations, as a whole, are intermediate to the parents in kernel size. F<sub>4</sub> families that varied widely in kernel weight were recombined. These data suggest that the inheritance of kernel weight is conditioned by multiple factors.

## TEST WEIGHT

Test weight is an important component of wheat quality since it generally reflects flour yield. To study its mode of inheritance test-weight determinations were made on wheat from hybrid populations and parent rows grown under comparable environmental conditions. The data, arranged in frequency distributions, are shown in table 7.

TABLE 7.—Frequency distribution of test weight of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties

Parent or cross	Generation	Year	Lines in class center for test weight in number of pounds stated										Lines	Mean	Standard deviation
			54.5	55.5	56.5	57.5	58.5	59.5	60.5	61.5	62.5	63.5			
			No.	No.	No.	No.	No.	No.	No.	No.	No.	No.			
American Banner	P <sub>1</sub>	1938	2	4	3	9	3	1	---	---	---	---	22	56.9	1.24
Do.	P <sub>1</sub>	1940	---	---	3	7	9	3	---	---	---	---	22	58.1	.92
Trumbull	P <sub>1</sub>	1938	---	1	1	8	5	6	1	---	---	---	22	58.8	.83
Do.	P <sub>1</sub>	1940	---	---	---	3	1	12	6	---	---	---	22	59.5	.95
Michikof	P <sub>1</sub>	1938	---	---	1	2	2	7	7	3	---	---	22	60.0	1.29
Do.	P <sub>1</sub>	1940	---	---	---	1	1	6	10	2	3	---	22	60.5	1.07
AB $\times$ T	F <sub>3</sub>	1938	2	6	22	44	79	43	4	---	---	---	200	58.2	1.13
Do.	F <sub>3</sub>	1940	2	13	34	43	58	47	3	---	---	---	200	58.0	1.29
AB $\times$ M.	F <sub>3</sub>	1938	---	---	8	35	60	67	30	---	---	---	200	58.9	1.60
Do.	F <sub>3</sub>	1940	---	---	---	1	5	37	107	48	2	---	200	60.5	.78
T $\times$ M.	F <sub>3</sub>	1938	---	---	1	6	27	68	80	15	3	---	200	59.9	.99
Do.	F <sub>3</sub>	1940	---	---	---	1	12	74	95	18	---	---	200	61.1	.76
Pedigree No.:															
76	F <sub>4</sub>	1940	5	5	10	5	1	---	---	---	---	---	26	56.2	1.12
5	F <sub>4</sub>	1940	---	---	---	9	13	6	---	---	---	---	28	58.4	.74
289	F <sub>4</sub>	1940	---	---	---	---	1	9	17	2	1	---	30	60.3	.78
322	F <sub>4</sub>	1940	---	---	---	---	---	---	16	3	---	---	30	61.2	.70
277	F <sub>4</sub>	1940	---	---	---	---	---	---	9	12	7	---	28	62.4	.78

Comparing the data of the two seasons, it is evident that the wheat of the 1938 crop was lower in test weight than that of the 1940 crop. The average test weights, for the 2 years, of American Banner, Trumbull, and Michikof, are 57.5, 59.2, and 60.2 pounds, respectively. The  $F_2$  distributions of test weight, for each of the 3 hybrid populations, suggest normal curves.  $F_4$  families varying from 56.2 to 62.4 pounds in test weight were selected. The data indicate, therefore, that test weight is a quantitative character governed to some extent by several genetic factors.

#### COLD RESISTANCE

In studying the inheritance of cold resistance,  $F_3$  and  $F_4$  lines, together with their parents, were subjected to artificially controlled freezing tests during the winters of 1938-39 and 1939-40. The data obtained are presented in table 8.

TABLE 8.—Frequency distribution of cold resistance of hybrids from American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses and of the parent varieties, as determined by controlled freezing tests

Parent or cross	Generation	Lines in class center for estimated winter survival in percentage stated									Lines	Mean	Standard deviation
		17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5			
American Banner.....	P <sub>1</sub>	No. 19	No. 31	No. 18	-----	-----	-----	-----	-----	-----	No. 50	Pct. 20.6	2.42
Trumbull.....	P <sub>1</sub>	1	31	18	-----	-----	-----	-----	-----	-----	50	24.2	2.61
Michikof.....	P <sub>1</sub>	1	31	18	-----	-----	1	17	29	3	50	50.9	3.11
AB × T.....	F <sub>3</sub>	35	136	29	-----	-----	-----	-----	-----	-----	200	22.4	2.83
AB × M.....	F <sub>3</sub>	2	14	37	51	53	32	10	1	-----	200	34.5	6.78
T × M.....	F <sub>3</sub>	-----	1	11	25	65	57	28	13	-----	200	38.0	6.26
Pedigree No.:													
509.....	F <sub>4</sub>	16	3	-----	-----	-----	-----	-----	-----	-----	19	18.3	1.88
380.....	F <sub>4</sub>	3	11	10	3	-----	-----	-----	-----	-----	27	24.9	4.27
219.....	F <sub>4</sub>	-----	2	12	9	5	-----	-----	-----	-----	28	30.5	4.42
635.....	F <sub>4</sub>	-----	-----	1	12	5	3	-----	-----	-----	21	34.9	4.09
339.....	F <sub>4</sub>	-----	-----	-----	-----	1	5	16	4	-----	26	46.9	4.06

The results show an average estimated winter survival of 20.6, 24.2, and 50.9 percent for the parental varieties American Banner, Trumbull, and Michikof, respectively.  $F_3$  families varied in cold resistance from that of the tender to that of the hardier parent, with the majority of the lines intermediate to the parents. Some  $F_4$  lines were recovered that were as susceptible to frost injury as the nonhardy variety American Banner, while others were almost as hardy as Michikof.

The increase in the variability between the parents and the  $F_3$  generation indicates the segregation of genetic factors. The results indicate, therefore, that cold resistance is a quantitative character conditioned by several genetic factors. These results confirm the findings of other investigators, especially those of Hayes and Aamodt (26), Nilsson-Ehle (33), Quisenberry (34), and Worzella (44).

#### MORPHOLOGICAL CHARACTERS

Since the parental varieties varied in several morphological characters, records were made of the colors of the straw, coleoptile, glume, and kernels of the hybrid materials. On the basis of  $F_3$  breeding behavior, the families were classified as homozygous dominant,

heterozygous, or homozygous recessive. Since the development of straw color was influenced considerably by environmental conditions, some difficulty was experienced in distinguishing between bleached purple and white culms. Consequently, the homozygous dominant and heterozygous families for straw color were combined. The results were summarized and the  $F_2$  segregation of the morphological characters in three crosses is given in table 9.

It will be noted from the data in table 9 that the hybrids segregated

TABLE 9.— $F_2$  segregation of morphological characters in American Banner  $\times$  Trumbull, American Banner  $\times$  Michikof, and Trumbull  $\times$  Michikof crosses

Parents and characters	Ratio	Number of families in class indicated			$\chi^2$	P
		Homozygous dominant	Heterozygous	Homozygous recessive		
American Banner $\times$ Trumbull:						
Brown and white glume.....	{Observed.....	49	102	49	0.08	0.96
	{Calculated (1:2:1)....	50	100	50		
Red and white kernel.....	{Observed.....	53	97	50	.27	.89
	{Calculated (1:2:1)....	50	100	50		
Purple and green coleoptile....	{Observed.....	50	107	43	1.47	.48
	{Calculated (1:2:1)....	50	100	50		
Purple and white straw.....	{Observed.....	157		43	1.31	.26
	{Calculated (3:1).....	150		50		
American Banner $\times$ Michikof:						
Brown and white glume.....	{Observed.....	50	105	45	.75	.73
	{Calculated (1:2:1)....	50	100	50		
Red and white kernel.....	{Observed.....	196		4	.27	.68
	{Calculated 63:1).....	196.9		3.1		
Trumbull $\times$ Michikof:						
Purple and green coleoptile....	{Observed.....	47	103	50	.27	.89
	{Calculated (1:2:1)....	50	100	50		
Purple and white straw.....	{Observed.....	150		50	0	1.00
	{Calculated (3:1).....	150		50		

in a 1:2:1 ratio for glume, coleoptile, and kernel color, and a 3:1 ratio for straw color. These ratios indicate a single-factor difference for each of the characters in the crosses studied. Also, in the American Banner  $\times$  Michikof cross, three pairs of factors are involved in the inheritance of kernel color.

#### INTERANNUAL CORRELATIONS

Interannual correlation coefficients were calculated for the components of wheat quality in order to learn the extent to which the hybrids reacted in the same way in different seasons. These data are reported in table 10.

It will be noted that highly significant positive interannual correlation coefficients were obtained for the components of quality studied. Since the soft wheat varieties, American Banner and Trumbull, vary slightly in quality, hybrids from this cross show correlation coefficients of lower magnitude than those obtained on the more diverse hybrids from crosses involving hard and soft wheats.

The high coefficients of correlation for gluten strength, granulation, and carotenoid content, especially in the American Banner  $\times$  Michikof and Trumbull  $\times$  Michikof crosses, indicate that the hybrids reacted in much the same way in the two seasons. The magnitude of the interannual correlations for protein, 1,000-kernel weight, and test weight, although highly significant, is rather low, indicating that environmental conditions greatly influence the expression of these characters.



TABLE 10.—*Interannual correlation coefficients of components of wheat quality of 200 hybrids from each of American Banner × Trumbull, American Banner × Michikof, and Trumbull × Michikof crosses grown during 1938 and 1940*

Components of quality	Correlation coefficients on the data obtained from the 1938 and 1940 crops in <sup>1</sup> —		
	American Banner × Trumbull	American Banner × Michikof	Trumbull × Michikof
Gluten strength.....	0.49	0.79	0.77
Granulation.....	.47	.84	.89
Carotenoid content.....	.28	.76	.82
Protein.....	.30	.35	.37
1,000-kernel weight.....	.47	.65	.53
Test weight.....	.24	.42	.49

<sup>1</sup> A correlation coefficient of 0.181 is at the 1-percent level of significance.

The consistency and magnitude of the interannual correlation coefficients for each of the three crosses provides further evidence that the variance observed in the characters gluten strength, granulation, carotenoid content, protein, kernel weight, and test weight in wheat is, in part, genetic in nature.

#### INTERRELATIONSHIP BETWEEN CHARACTERS

Many wheat investigators are of the opinion that associations exist between certain components of quality and also between certain of these and winter hardiness. For example, (1) hard vitreous wheats are regarded as being high in protein content and possessing a strong gluten, while soft starchy kernels are considered as being low in protein and weak in gluten strength, (2) white wheats are believed to possess weaker gluten than redkernel wheats- and (3) soft wheats are regarded as less winter hardy than hard wheats. These relationships are based, for the most part, on observations and experiments conducted on a series of varieties or selected hybrid strains. Associations between characters obtained from such studies would be affected by the wheats chosen, and would not necessarily reflect the natural relationships present in randomly selected hybrid populations.

For the present investigations 200 hybrid families from each cross, selected at random, were used. Obviously, such a sample represents a population of wheats in which the recombination of characters was governed by the laws of heredity. In determining the relationships between the 11 characters studied, the data were subjected to correlation, chi-square, and linkage analysis.

#### CORRELATION AND CHI-SQUARE ANALYSIS

To measure the degree of association, if any, the method of correlation was used for the quantitative characters, while the chi-square test was used where qualitative characters were involved. Since the hybrids of the three crosses represent different populations, correlation and chi-square analysis were calculated separately for each cross. The data, indicating the interrelationship of components of quality, cold resistance, and several morphological characters of wheat hybrids from three crosses, are reported in tables 11, 12, and 13.

The intercharacter correlations in tables 11, 12, and 13, indicate

very little, if any, association between cold resistance and the components of quality studied. Although several of the correlation coefficients are statistically significant, they are rather small and inconsistent in each of the three crosses and for the 2 crop years. The morphological characters kernel color, glume color, coleoptile color, and straw color, are inherited independently of cold resistance.

TABLE 11.—Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from American Banner  $\times$  Trumbull cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities <sup>1</sup>

Characters	Crop of—	Association between characters indicated <sup>2</sup>								
		Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight	Kernel color	Glume color
Gluten strength	1938	0.07								
	1940	-.12								
Granulation	1938	.11	0.07							
	1940	.06	.26**							
Carotenoid content	1938	-.04	-.17*	0.38**						
	1940	-.10	-.02	-.01						
Protein	1938	.09	.13	-.08	-.08					
	1940	.08	.28**	-.04	-.11					
1,000-kernel weight	1938	.06	.12	-.30**	-.53**	-.10				
	1940	-.02	.04	-.06	-.39**	.03				
Test weight	1938	.12	.02	-.17*	-.33**	-.12	0.42**			
	1940	.10	-.02	.23**	-.17*	-.10	.17*			
Kernel color	1938	71	54	13	76	<1**	61	61		
	1940	71	85	40	10	<1**	79	56		
Glume color	1938	24	<1**	70	49	77	6	46	78	
	1940	24	.2*	30	97	20	81	75	53	
Coleoptile color	1938	80	28	95	77	82	15	60	83	87
	1940	80	75	74	50	78	60	86	26	72
Straw color	1938	80	28	95	77	82	15	60	83	87
	1940	80	75	74	50	78	60	86	26	72

<sup>1</sup> Percentage probability for chi-square test.

<sup>2</sup> \*Odds at least 19 to 1; \*\*odds at least 99 to 1.

TABLE 12.—Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from American Banner  $\times$  Michikof cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities <sup>1</sup>

Characters	Crop of—	Association between characters indicated <sup>2</sup>						
		Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight
Gluten strength	1938	0.10						
	1940	.08						
Granulation	1938	-.17*	0.01					
	1940	-.09	.03					
Carotenoid content	1938	-.04	-.08	0.16*				
	1940	.03	.14*	.17*				
Protein	1938	-.08	.24**	.16*	0			
	1940	-.15*	.26**	-.01	-.02			
1,000-kernel weight	1938	.22**	.15*	-.20**	-.10	0.01		
	1940	.02	.02	-.10	-.17*	.16*		
Test weight	1938	.05	.18*	-.12	-.16*	.10	0.33**	
	1940	-.10	.05	-.04	-.06	.02	.15*	
Glume color	1938	98	<1**	47	73	73	44	27
	1940	98	<1**	91	85	62	40	84

<sup>1</sup> Percentage probability for chi-square test.

<sup>2</sup> \*Odds at least 19 to 1; \*\*odds at least 99 to 1.

TABLE 13.—Relations among components of quality, cold resistance, and morphological characters of 200 wheat hybrids from Trumbull  $\times$  Michikof cross grown during 1938 and 1940, expressed by correlation coefficients and chi-square probabilities<sup>1</sup>

Characters	Crop of—	Association between characters indicated <sup>2</sup>							
		Cold resistance	Gluten strength	Granulation	Carotenoid content	Protein	1,000-kernel weight	Test weight	Coleoptile color
Gluten strength.....	{ 1938	-.17*							
	{ 1940	-.07							
Granulation.....	{ 1938	-.10	-.07						
	{ 1940	-.06	-.05						
Carotenoid content.....	{ 1938	-.10	-.02	0.33**					
	{ 1940	-.13	.14*	.13					
Protein.....	{ 1938	-.22**	.03	.07	-.01				
	{ 1940	.07	.02	-.04	-.13				
1,000-kernel weight.....	{ 1938	-.11	-.07	.09	-.17*	-.09			
	{ 1940	.04	.16*	.15*	-.13	.06			
Test weight.....	{ 1938	.09	-.04	-.09	-.14*	-.09	0.31**		
	{ 1940	-.06	.13	-.28**	-.16*	.27**	.11		
Coleoptile color.....	{ 1938	41	93	38	58	2*	55	19	
	{ 1940	41	34	70	53	37	59	61	
Straw color.....	{ 1938	41	93	38	58	2*	55	19	0**
	{ 1940	41	34	70	53	37	59	61	0**

<sup>1</sup> Percentage probability for chi-square test.

<sup>2</sup> \*Odds at least 19 to 1; \*\*odds at least 99 to 1.

The data indicate that in these hybrid populations, at least, the character gluten strength appears to be inherited independently of the other components of quality. However, highly significant correlation coefficients were obtained between gluten strength and protein content in the American Banner  $\times$  Michikof cross (table 12). The results indicate that the genes controlling kernel color, coleoptile color, and straw color were not linked with those for gluten strength.

The positive correlation coefficients obtained between granulation and carotenoid content suggest that these characters are associated. It is well known, however, that granulation or fineness of the wheat meal greatly influences the extraction of the carotenoid pigments. It appears probable, therefore, that the correlations obtained between granulation and carotenoid content are due to the limitations of the grinding methods in the preparation of meal of comparable fineness, rather than to genetic linkage. Granulation appears to be correlated negatively with test weight, however; the coefficients are either small or nonsignificant. Very little, if any, relationship was found between granulation and the other quality factors and morphological characters.

Carotenoid pigment content was found to be correlated negatively with 1,000-kernel weight and test weight. Also, 1,000-kernel weight was correlated positively with test weight. It appears, therefore, that hybrids possessing large kernels that are high in test weight, tend to be lower in carotenoid content and vice versa. The relationships between these characters, however, are not close, since most of the coefficients are low in magnitude and others are not significant.

Chi-square analysis of the data reveals that the genes for the following characters are linked: (1) gluten strength and glume color, (2) protein content and kernel color, and (3) coleoptile color and straw color.

Genetic data reported in table 9 indicate that the inheritance of coleoptile and straw color is monogenic. Pedigree records reveal that, without exception, all of the hybrids were classified as either purple coleoptile with purple straw or green coleoptile with white straw. If 2 independent pairs of genetic factors were involved, one would expect a double recessive combination once out of every 16  $F_3$  lines. None were found in the 400 families examined. It appears that the inheritance of coleoptile and straw color is governed either by 2 pairs of genetic factors closely linked, or by the same pair of genes.

#### LINKAGE OF GLUTEN STRENGTH AND GLUME COLOR

Data reported in tables 11 and 12 show an association between the quantitative character gluten strength and the qualitative character glume color. This relation suggests a genetic linkage between a single pair of genes for glume color and one or more pairs of genes for gluten strength. Since in the American Banner  $\times$  Trumbull cross, the inheritance of both characters, glume color and gluten strength, was found to be monogenic (table 2, fig. 1), it was possible to calculate their linkage intensity. The percentage crossing over was calculated on the  $F_2$  segregation by the product method formula. The data are shown in table 14.

TABLE 14.—Linkage relation between glume color (*Gc gc*) and gluten strength (*G g*) in American Banner (*Gc g*) (*Gc g*)  $\times$  Trumbull (*gc G*) (*gc G*) cross

Item	Crop year	Number of families having phenotype—				$\chi^2$	<i>P</i>
		<i>Gc G</i>	<i>Gc g</i>	<i>gc G</i>	<i>gc g</i>		
Actual.....	1938	102	49	44	5		
Calculated, 30.5 percent crossing over.....	1938	104.6	45.4	45.4	4.6	0.42	0.85
Actual.....	1940	110	41	42	7		
Calculated, 38.8 percent crossing over.....	1940	107.5	42.5	42.5	7.5	.15	.93

It will be noted that approximately 30.5 percent and 38.8 percent crossing over was observed between the pair of genes for glume color and that governing the inheritance of gluten strength for the 1938 and 1940 crop years, respectively. The results of the 2 years agree rather closely, considering that these characters appeared in the repulsion phase and that only 200 hybrids were used.

#### LINKAGE OF PROTEIN CONTENT AND KERNEL COLOR

In table 11 it was noted that, in the hybrids originating from the American Banner  $\times$  Trumbull cross, the characters protein content and kernel color were associated. The protein data reported in table 5 and figure 4 suggest that probably a single pair of genetic factors controls the inheritance of this character in the American Banner  $\times$  Trumbull cross. Accordingly, arbitrary classes for protein content were set up, and the hybrids classified into three groups approaching a 1:2:1 ratio. Table 15 reports the  $F_2$  distribution and percentage crossing over for protein content and kernel color in the American Banner  $\times$  Trumbull cross.



TABLE 15.—Linkage relation between kernel color (*R r*) and protein content (*N n*) in American Banner (*r n*) (*r n*) × Trumbull (*R N*) (*R N*) cross

Item	Crop year	Number of families having phenotype—				$\chi^2$	P
		<i>R N</i>	<i>R n</i>	<i>r N</i>	<i>r n</i>		
Actual	1938	130	20	24	26		
Calculated, 25.7 percent crossing over	1938	127.6	22.4	22.4	27.6	0.51	0.81
Actual	1940	127	23	28	22		
Calculated, 30.9 percent crossing over	1940	123.9	26.1	26.1	23.9	.79	.71

The results in table 15 show that about 25.7 percent and 30.9 percent crossing over occurred between the pair of genes for protein content and that for kernel color for the 1938 and 1940 crop years, respectively. The results of the 2 years agree closely, considering that protein content is greatly influenced by environmental conditions.

#### SUMMARY

Experiments were conducted on 600  $F_3$  and  $F_4$  hybrids, and 66 parent rows originating from a triangular cross of American Banner, Trumbull, and Michikof wheats, grown at Lafayette, Ind., in 1937–38 and repeated in 1939–40.

The inheritance and interrelationship of components of quality, cold resistance, and several morphological characters were studied in randomly selected hybrid populations.

The mode of inheritance of gluten strength, granulation, carotenoid content, crude protein, kernel weight, test weight, and cold resistance was found to be quantitative and governed by several genetic factors. In the hybrids originating from the American Banner × Trumbull cross, the inheritance of gluten strength and protein content appeared to be monogenic.

Single genetic-factor differences were found to govern the mode of inheritance of the characters glume, coleoptile, and straw color. The inheritance of kernel color was governed by one and three pairs of genes in the crosses studied.

Highly significant interannual correlation coefficients were obtained for the components of quality studied. The consistency and high magnitude of the correlations provide further evidence that the variance observed in the characters gluten strength, granulation, carotenoid content, protein content, kernel weight, and test weight in wheat is, in part, genetic in nature.

Intercharacter correlations indicate very little, if any, association between cold resistance and the components of quality studied.

Few relationships were found between components of wheat quality in hybrid material selected at random. In general, for most of the characters, the coefficients were low in magnitude or not significant. Gluten strength and granulation were inherited independently of the other components of quality. However, granulation was correlated negatively with test weight. Carotenoid content was correlated negatively with kernel weight and test weight, while kernel weight was correlated positively with test weight.

Genetic linkage was found between the genes for the following characters: (1) gluten strength and glume color, (2) protein content and kernel color, and (3) coleoptile color and straw color.

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# THE RESPONSE OF "CEASED" REACTORS IN BANG'S DISEASE TO REEXPOSURE<sup>1</sup>

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## INTRODUCTION

The results of observations on cattle under controlled conditions, and also in the field, agree in supporting the conclusion that infection with a virulent strain of *Brucella abortus* usually induces resistance to subsequent infection by the organism, presumably as the result of active immunization. This phenomenon has long been recognized by some workers—among them Birch and Gilman (2)<sup>2</sup> and Giltner (9)—and has been questioned by others, particularly insofar as the possibility of complete recovery from the infection is concerned. Complete recovery would imply elimination of the organism from the animal's body and a return of the agglutination titer of the serum to that of a noninfected individual.

The observations reported in this paper were made on cows whose sera, following induced infections, had lost their agglutinin titer for the infecting organism. These observations were made on two groups of animals, in two different herds consecutively assembled, over a period from August 1933 through July 1939. In a preceding article (1), the explanation was made that not all the animals to which the term "ceased" reactor is applied, had been definite reactors according to the accepted standard. Since the principal point of interest in this paper is the reaction of all previously infected individuals to a second exposure, the terminology for these animals is unchanged.

## MATERIALS AND METHODS

The animals on which these observations were made were in two herds, as previously described (1).

Herd 1 originally consisted of 44 grade and purebred Holstein calves, and was assembled in 1926 as described by Hart and his coworkers (11). After the artificial exposure of 37 pregnant individuals in 1928, and subsequent observations on them for a period of 2 years following the exposure, 14 individuals were selected to constitute a part of herd 2. At that time the agglutinins for *Brucella abortus* had practically or entirely disappeared from the serum of each of these 14 animals, and they were therefore termed "ceased" reactors.

To these 14 cows from herd 1 were added 22 animals, mostly virgin heifers, to make up herd 2. It was thought that none of these 22 animals had had any previous contact with *Brucella abortus*, although

<sup>1</sup> Italic numbers in parentheses refer to Literature Cited, p. 531.

<sup>2</sup> Received for publication February 27, 1942. Contribution from the Department of Veterinary Science and the Department of Genetics (paper No. 281), Wisconsin Agricultural Experiment Station, and the Bureau of Animal Industry, U. S. Department of Agriculture.

it was later determined that one cow (No. 23A) had been in a herd which had suffered an outbreak of Bang's disease some years previously. The individuals in herd 2 were artificially exposed in September 1933 by instilling two drops of a heavy suspension of *Brucella abortus* into the eye of each.

Herd 3 was assembled in August 1934. This group consisted of 8 individuals (ceased reactors) from herd 2, and 28 virgin heifers which were purchased from herds free of Bang's disease. One of the ceased reactors developed acute mastitis early in the experiment and was removed. Other removals and substitutions were made, as will be described more fully later. All animals in the herd were artificially exposed to *Brucella abortus* in May 1937. Cultures of the same strains of the organism were used that had previously been found by Crawford and Beach (6) to possess a marked pathogenicity for susceptible cattle.

The serum of each of the animals in both of the herds used in these experiments (herds 2 and 3) was tested, usually monthly, often weekly, for its agglutinin titer against *Brucella abortus*. Further, at each parturition, the colostrum and either the placenta or the uterine fluid, or both, were tested for the presence of *Brucella abortus* by the usual method of inoculation of guinea pigs.

#### EXPERIMENTAL RESULTS

##### HERD 2

Data are presented in table 1 for the animals of herd 2. These include the pregnancies completed prior to the induced infection of all the animals of the herd in September 1933, and the subsequent abortion or normal calving of each individual. The first 14 individuals listed in the table were the so-called ceased reactors from herd 1. The others, with the possible exception of 23A as described above, presumably had had no previous contact with *Brucella abortus*.

TABLE 1.—Results obtained with the animals of herd 2 prior to and following their artificial exposure <sup>1</sup> to *Brucella abortus*

Cow No.	Preexposure period			Postexposure period				<i>Brucella abortus</i> recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		<i>Brucella abortus</i> re-covered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
4.....	2	0	No.....	26	283	257	No.....	No.....	No.....	<1:25	20
5.....	1	0	No.....	87	152	65	Yes.....	Yes.....	No.....	1:200	10
8.....	2	0	No.....	( <sup>2</sup> )					No.....	<sup>3</sup> 1:100	17
12.....	2	0	No.....	97	283	186	No.....	No.....	No.....	1:25	19
17.....	2	0	No.....	30	279	249	No.....	No.....	No.....	<1:25	13
18.....	2	0	No.....	( <sup>4</sup> )							
28.....	2	0	No.....	78	287	209	No.....	No.....	No.....	1:50	19
31 <sup>5</sup> .....	1	0	No.....	79	279	200	No.....	No.....	No.....	<1:25	15
33.....	2	0	No.....	94	283	189	No.....	No.....	No.....	<1:25	19
35.....	2	0	No.....	73	287	214	No.....	No.....	No.....	1:25	19
36.....	2	0	( <sup>6</sup> )	82	281	199	No.....	No.....	No.....	1:50	15
38.....	2	0	No.....	70	279	209	No.....	No.....	No.....	1:100	15
40.....	2	0	No.....	89	283	194	No.....	No.....	No.....	<1:25	19

See footnotes at end of table.

TABLE 1.—Results obtained with the animals of herd 2 prior to and following their artificial exposure<sup>1</sup> to *Brucella abortus*—Continued

Cow No.	Preexposure period			Postexposure period				Brucella abortus recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		Brucella abortus recovered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
44.....	2	0	No....	96	281	185	No....	No....	No....	>1:25	15
5A.....	1	0	No....	(4)							
6A.....	2	0	No....	(4)							
7A.....	2	0	No....	(4)							
23A.....	2	0	No....	36	281	245	No....	No....		1:25	15
25A.....	2	0	No....	45	211	166	Yes	Yes	Yes...	1:400	15
28A.....	2	0	No....	26	196	170	Yes	Yes	Yes...	1:400	13
32A.....	2	0	No....	46	280	234	No....	No....		1:100	10
55A.....				34	235	201	Yes	No....		1:50	8
61A.....				56	164	108	Yes	Yes	Yes...	1:400	15
63A.....				77	287	364	No....	No....		1:25	4
1B.....	2	0	No....	89	181	92	Yes	Yes	No....	1:200	13
2B.....	2	0	No....	49	164	115	Yes	No....	No....	1:25	4
3B.....	2	0	No....	57	169	112	Yes	Yes	Yes...	1:100	15
9B.....	1	0	No....	7	278	271	No....	No....	No....	1:25	10
10B.....	2	0	No....	91	179	88	Yes	No....		1:50	8
11B.....	(7)	0	No....	(4)							
13B.....	2	0	No....	79	167	88	Yes	Yes	No....	1:400	15
14B.....	1	0	No....	(2)					No....	1:400	10
15B.....	2	0	No....	12	290	278	No....	No....	No....	1:100	8
16B.....	2	0	No....	29	284	313	No....	No....	No....	1:100	15
19B.....	1	0	No....	101	199	98	Yes	Yes	Yes...	1:400	19
20B.....	2	1	No....	2	232	230	(5)	Yes	Yes...	1:400	15
21B.....	1	0	No....	85	175	90	Yes	Yes	Yes...	1:400	13
22B.....	2	0	No....	(2)					No....	1:100	13
24B.....	2	0	No....	(2)					No....	1:400	14

<sup>1</sup> Two drops of a heavy suspension of *Br. abortus* were instilled into an eye of each animal listed as exposed.

<sup>2</sup> Exposed but not pregnant.

<sup>3</sup> S=slight reaction.

<sup>4</sup> Not exposed.

<sup>5</sup> This cow aborted in April 1931, 4 months before herd 2 was assembled. No *Br.* organisms were recovered at that time.

<sup>6</sup> *Br. abortus* was isolated from the colostrum of the left rear quarter of the udder at calving on October 30, 1932. The serum of the colostrum was positive (1:100) in all quarters. No organisms were isolated at the next calving.

<sup>7</sup> This heifer became a reactor late in November 1931, and was removed from the herd. She calved normally on May 23, 1932. No organisms were recovered at the time of calving.

<sup>8</sup> A premature but living calf was produced.

As will be noted from the data in table 1 (columns 2, 3, and 4),<sup>1</sup> all calvings previous to the exposure were normal and the pathogen of Bang's disease was not isolated from any individual, except from the colostrum of cow 36 (a ceased reactor) in October 1932, and at the one calving only. Furthermore, although there were slight fluctuations in the agglutinin titer of the sera from the ceased reactors, these were never higher than suspicious reactions.

Shortly after the herd was assembled, the serum of cow 11B reacted with the organism at a dilution of 1:100, and she was immediately removed from the herd and isolated. Although *Brucella abortus* was not recovered following birth at full term of a normal calf, the probability that she was infected cannot be denied. The only animal which, within the writers' knowledge, might have been a carrier of the organism was No. 36, but whether the presumed infection of No. 11B came from this particular cow, or was latent at the time of purchase, cannot be stated definitely.

Four animals (18, 5A, 6A, and 7A) were taken out of the herd, and three (58A, 61A, and 63A) were added about 3 months before the exposure. Each of the three replacements had calved shortly before they were placed in herd 2, and came from a herd free of Bang's disease.

There were 34 cows in the herd at the time of the artificial exposure on September 5, 1933. Of these, 13 were ceased reactors, and 21 were normal individuals, except for 23A, as previously described. These were given by way of the eye two drops of a heavy suspension of *Brucella abortus*. Also, a reacting cow which had recently aborted was put in with these animals to allow further exposure by contact. Four of these animals (8, 14B, 22B, and 24B) presumably were not pregnant at the time of exposure. The serum of one of these (No. 8) showed no appreciable increase in agglutinin titer following exposure, while that of the others gave definite evidence of infection; the agglutination titers of their respective sera increased markedly after the exposure, as shown in table 1.

Only one abortion was noted (No. 5) in the group of ceased reactors which were pregnant when exposed. This cow became a definite reactor at approximately 8 weeks after the exposure, and aborted a fetus on the sixty-fifth day after inoculation. *Brucella abortus* was demonstrated in the material examined. A post-mortem examination when this animal was killed, approximately 2 months after the abortion, revealed a severe (pus) infection of both kidneys. Whether this infection was a major contributing factor in the break-down of whatever immunity this cow possessed as a result of the first infection, is not definitely known. About 2 months after the date of the first exposure in 1928 (11) the serum of this animal produced a slight agglutination of the organism at a dilution of 1:100, and the organism was not recovered at the time of calving, nor thereafter. It cannot therefore be stated definitely that this animal was not infected at the first exposure, although that may be the explanation for the abortion following the second infection when at least some degree of resistance would have been anticipated as a result of the previous exposure.

One of the ceased reactors (No. 8) presumably did not conceive. Each of the other 11 cows of this group (4, 12, 17, 28, 31, 33, 35, 36, 38, 40, and 44) of ceased reactors produced living calves at full term, and *Brucella abortus* was not recovered from any of them. Only 3 of these 11 cows (28, 36, and 38) showed even suggestive evidence of infection, as indicated by a slight increase in the agglutinating titer of their respective sera for the organism. The sera of the other 8 cows only occasionally, if ever, produced complete agglutination of the organism at a dilution of 1 in 25.

Thus it would appear that although the first exposure (11) of these 12 cows was made 5 years before the second, the resistance induced in each by the first infection was sufficient to protect against a subsequent exposure, except for 1 individual (No. 5). Each of these cows had produced either 3 or 4 calves following the calving or abortion subsequent to the initial exposure, and prior to the second.

As stated above, cow 23A was in a herd which, several years previously, had suffered an outbreak of Bang's disease. This herd, however, was free of the disease previous to her transfer to this experiment. The entire lack of any symptoms on the part of this



animal (i. e., no increase in agglutinins and no organisms recovered at calving) subsequent to exposure aroused suspicion, and a reexamination of the records of the herd from which she came elicited the above information.

Excluding this individual, among the group of 17 susceptible cows there were 5 which calved normally, 11 which aborted their fetuses at various stages of pregnancy, and one (20B) which produced a living but premature calf. *Brucella abortus* was not recovered at calving from any of the 5 which carried their calves full term; 3 of these (32A, 15B, and 16B) were definite but low reactors at serum dilutions of 1:100, and the sera of the other two (63A and 9B) never produced agglutination at a dilution higher than 1:25.

In the group of susceptible animals, only 1 (2B) of 11 individuals which aborted showed a serum reactivity less than that considered as indicative of a definite reactor. All others were definitely reactors. The organism was not recovered from 3 of these (58A, 2B, and 10B) by the usual guinea pig inoculations following the respective abortions. It is therefore possible that the abortion of 2B was not due to *Brucella abortus*, although it is assumed that the abortions of the other 10 individuals were due to the pathogen.

It is of interest to note that two cows (63A and 16B), which were bred for the final service after the induced exposure, calved normally. Edgington and Donham (8) have recently reported, with greater numbers, similar observations, to the effect that cows infected prior to pregnancy are not likely to abort.

At the time of slaughter of these animals, minced tissues from the supramammary lymph gland, minced udder tissue, and washings from the udder of each were injected into guinea pigs in an attempt to determine whether *Brucella abortus* was present. As will be seen in table 1, the organism was not found in all the individuals from which it had previously been recovered at abortion or calving. However, it was found at slaughter only in animals from which it had been recovered at abortion or calving.

#### HERD 3

Approximately 8 months after the exposure of the cows in herd 2, there were 8 individuals (23A, 32A, 58A, 63A, 9B, 10B, 15B, and 16B) from the previously susceptible group whose sera showed either very low titers or no agglutinins for *Brucella abortus*. The sera of 3 of these (23A, 63A, and 9B) had shown no reactions with complete agglutination of the organisms at a dilution higher than 1:25; the sera of the others had produced agglutination, but at relatively low dilutions. The other animals of the herd were disposed of, the barn and premises were thoroughly cleaned and disinfected, and 28 virgin heifers were added in August 1934 to make herd 3. Cow 16B developed acute mastitis and was removed a few weeks after herd 3 had been assembled.

Table 2 (columns 2 and 3) gives the number of normal pregnancies or abortions for each individual in the herd from August 1934 until the artificial exposure in May 1937. Five individuals (4C, 5C, 17C, 19C, and 21C) were exchanged in April 1936 for five others (19D, 22D, 33D, 38D, and 42D) from another experiment within the station, and six animals (16C, 22C, 26C, 31C, 33C, and 19D) were discarded

for various reasons, none of which was attributable to infection with *Brucella abortus*. Three heifer calves (3H, 4H, and 5H from cows 27C, 63A, and 32A, respectively) which reached sexual maturity prior to the artificial exposure, were bred and then exposed with the others, and data are given concerning their subsequent reactions.

TABLE 2.—Results obtained with the animals of herd 3 prior to and following their artificial exposure<sup>1</sup> to *Brucella abortus*.

Cow No.	Preexposure period			Postexposure period				<i>Brucella abortus</i> recovered—		Agglutination reaction and time observed	
	Number of pregnancies completed		<i>Brucella abortus</i> recovered	Days pregnant—		Days from exposure to abortion or calving	Aborted	At calving or abortion	Following slaughter	Highest titer	Weeks following exposure
	Normal	Abortion		When exposed	When aborted or calved						
23A.....	2	0	No.	72	( <sup>2</sup> )						
32A.....	2	0	No.	69	280	211	No.	No.	No.	( <sup>3</sup> ) 50	2
58A.....	2	0	No.		( <sup>2</sup> )					50	2
63A.....	2	0	No.		( <sup>2</sup> )					50	2
9B.....	3	0	No.	80	281	201	No.	Yes	No.	50	2
10B.....	2	0	No.	80	275	195	No.	No.	No.	50	2
15B.....	2	0	No.	91	278	187	No.	No.	No.	100	2
1C.....				( <sup>4</sup> )							
2C.....	2	0	No.	65	134	69	Yes.	Yes.	No.	400	5
3C.....	2	0	No.	69	181	114	Yes.	Yes.	No.	400	16
4C.....	1	0	No.	( <sup>5</sup> )							
5C.....	1	0	No.	( <sup>5</sup> )							
6C.....	2	0	No.	84	188	104	Yes.	Yes.	Yes.	400	5
7C.....	1	1	No.	30	179	149	Yes.	Yes.	Yes.	400	9
8C.....	2	0	No.	64	110	46	Yes.	Yes.	Yes.	400	12
11C.....	2	0	No.	90	183	93	Yes.	Yes.	Yes.	400	5
12C.....	2	0	No.	83	173	90	Yes.	Yes.	No.	200	14
13C.....	2	0	No.	93	191	98	Yes.	Yes.	Yes.	400	3
14C.....	1	0	No.	76	138	62	Yes.	Yes.	Yes.	400	9
16C.....	2	0	No.	( <sup>5</sup> )							
17C.....	1	0	No.	( <sup>5</sup> )							
18C.....	1	1	No.	34	240	206	( <sup>6</sup> )	Yes.	Yes.	400	11
19C.....	1	0	No.	( <sup>5</sup> )							
20C.....	2	0	No.	68	281	213	No.	Yes.	No.	( <sup>3</sup> )	
21C.....	1	0	No.	( <sup>5</sup> )							
22C.....	0	0	No.	( <sup>5</sup> )							
24C.....	2	0	No.	81	135	54	Yes.	Yes.	No.	200	4
25C.....	2	0	No.	94	202	108	Yes.	Yes.		400	10
26C.....	1	1	No.	( <sup>5</sup> )							
27C.....	2	0	No.	71	281	210	No.	Yes.		400	16
28C.....	2	0	No.	13	212	199	Yes.	Yes.	Yes.	400	9
29C.....	2	0	No.	79	213	134	Yes.	Yes.	Yes.	400	9
30C.....	2	0	No.	80	202	122	Yes.	Yes.	Yes.	400	17
31C.....	1	0	No.	( <sup>5</sup> )							
33C.....	1	0	No.	( <sup>5</sup> )							
19D.....	1	0	No.	( <sup>5</sup> )							
22D.....	1	0	No.	65	268	203	No.	Yes.	Yes.	200	10
33D.....	1	0	No.	58	187	129	Yes.	Yes.		400	18
38D.....	1	0	No.	178	251	73	Yes.	Yes.		200	8
42D.....	1	0	No.	( <sup>2</sup> )							
3H.....				71	186	115	No.	Yes.	Yes.	400	9
4H.....				18	270	252	No.	No.	No.	100	8
5H.....				61	191	130	Yes.	Yes.	Yes.	400	12

<sup>1</sup> Cows 23A, 32A, 58A, 63A, 9B, 10B, 15B, 14C, and 38D were exposed by way of the eye; the others of the herd were given per os 20 cc. of a heavy suspension of *Br. abortus*.

<sup>2</sup> Exposed but not pregnant.

<sup>3</sup> No reaction.

<sup>4</sup> Not exposed; see text.

<sup>5</sup> Not exposed.

<sup>6</sup> Small calf, lived 9 days.

There were 31 different cows which, prior to the induced exposure, had produced either 1 or 2 calves each while in contact with the 7 cows that had recovered from Bang's disease. Within this group and

during this period there were 3 abortions (7C, 18C, and 26C), but these presumably were not due to *Brucella abortus* since the organism was not detected in any of the 3, nor were there demonstrable agglutinins in their sera at a titer above 1:25.

At the time of the artificial exposure in May 1937 there were 31 individuals in the herd, including the 3 heifers mentioned. The 7 ceased reactors and 2 of the susceptible group (14C and 38D) were exposed by instilling 2 drops of a heavy suspension of *Brucella abortus* into the eye. The remaining susceptible animals were given per os 20 cc. of a heavy suspension of the same organism. The adequacy of the infection is attested by the fact that, of the 24 animals of the susceptible group, 19 aborted. There were 4 which produced normal calves at full term, and 1 (42D) presumably was not pregnant. The results of the guinea-pig inoculations with colostrum and uterine exudates at the time of calving or abortion, as given for each animal in table 2, show that the organism was present in each of the 19 susceptible animals that aborted, and also in 3 of the 4 individuals that calved normally.

Four of the seven cows of the ceased reactors produced calves, and these were all carried to full term. *Brucella abortus* was recovered from the uterine fluid of only one of these, cow 9B. The sera from two cows that did not produce calves (58A and 63A) showed a slight rise in agglutination titers very shortly after the exposure.

After slaughter, tissues from 23 of the infected animals were examined for the presence or absence of *Brucella abortus*. The same technique was used as was described for animals of herd 2; i. e., minced tissues from the supramammary lymph gland, from the udder, and washings from the udder were injected into guinea pigs. These tests were made during a period of 21 to 27 months following the exposure. As was found in comparable tests on the individuals of herd 2, not all the animals harbored the organisms which had shed them previously at the respective calvings or abortions. But the organisms were found in the tissues of only those animals from which they had previously been recovered.

An observation of considerable interest was made following the exposure. One cow (No. 1C) calved shortly before the other animals were exposed in May 1937, too late to be treated with them. This cow thereafter was stabled at one end of a row of stanchions which also held infected animals, but was never turned loose with them. During the summer and early fall months, she stood next to an infected cow for an hour or more two or three times a week. As the weather became cooler, all the cows were kept in the barn, except for a few hours a day when the infected animals were let loose in the yard.

No evidence of infection of 1C was noted until December 1937. The serum of this individual had been used weekly as a source of complement for bactericidal tests made on the infected animals, and a reaction out of the ordinary in these tests was noted on December 23, although no appreciable change in the agglutinin titer of the serum for *Brucella abortus* could be detected. Very shortly after this, however (on January 5, 1938), her serum showed a perceptible increase in agglutinins (giving complete agglutination at a dilution of 1:100), and it was learned that several days previous to December 23 this individual had been loose in the barn for several hours, thus

allowing contact with the infected animals. However, it cannot be stated definitely that the exposure was made only at this time. She was bred in February 1938, and produced a normal calf at full term, thus adding another to the examples given above, and to others cited by Edgington and Donham (8), that animals infected before pregnancy are likely to produce normal calves.

#### GENERAL CONSIDERATIONS

The combined results on the ceased reactors in herds 2 and 3 show that, of a total of 16 such individuals which were subjected to a second exposure, 15 produced normal calves at full term and 1 aborted. *Brucella abortus* was isolated at calving from the uterine exudate of only 1 of the 15 animals which produced the normal calves. Huddleson and his coworkers (12) were unable to detect the organism in 12 animals which had ceased to react. It appears probable, therefore, that the percentage of ceased reactors which may be found to harbor the organism will be low.

The comparative titers of the sera of the different ceased reactors to *Brucella abortus*, following the first and second exposures, respectively, have been summarized in table 3. The sera of six of these individuals showed an appreciably lower titer following the second exposure than was found during the first infection. There were nine animals, however, whose sera during the second infection showed difference in the height of the titer of only one dilution from that noted in the first infection, probably not a significant difference, and one individual (No. 5) which gave a higher agglutination titer following the second exposure. Most of this latter group of animals had never shown complete agglutination of the organism at a serum dilution higher than 1:100 during either the first or second infection.

TABLE 3.—A comparison of the agglutination titers for *Brucella abortus* of the sera of animals for the first and second infections,<sup>1</sup> respectively, with the pathogen

Cow No.	Highest titer during infections following—		Cow No.	Highest titer during infections following—	
	First exposure	Second exposure		First exposure	Second exposure
4.....	<sup>2</sup> S-1:100	S-1:100	36.....	1:10	1:50
5.....	S-1:100	1:200	38.....	1:200	1:100
12.....	1:200	1:25	40.....	S-1:200	1:25
17.....	1:200	S-1:100	44.....	1:100	S-1:100
28.....	1:100	1:50	32A.....	1:100	1:50
31.....	S-1:100	1:25	9B.....	1:25	1:50
33.....	1:200	1:25	10B.....	1:50	1:50
35.....	1:200	1:25	15B.....	1:100	1:100

<sup>1</sup> As a result of changes made in the sensitivity of the antigen over the years in which these tests were carried out, it seems probable that the respective agglutination titers for the sera from the different individuals recorded at the different times of testing are only relatively comparable.

<sup>2</sup> S indicates a slight reaction at the dilution shown.

The results obtained with the ceased reactors in the two different herds indicate that only a relatively small proportion of individuals exposed to a virulent culture of *Brucella abortus* immediately preceding or during pregnancy will be susceptible to a subsequent exposure. Furthermore, the demonstration (13) that the serum of the ceased reactors possessed bactericidal antibodies in the absence of agglutinins



beyond the titer of normal cattle adds support to the conclusion that an active immunity is engendered by an infection. Indirectly, then, all such data support the conclusions based upon experiments in vaccination with living cultures by various workers [Buck (3), Buck et al. (4), Cotton, Buck, and Smith (5), Delez (7), and Haring (10)] that vaccination induces a definite immunization in the treated animals.

#### SUMMARY

Only 1 of 16 ceased reactors in 2 different herds aborted its fetus when subjected to a second exposure. Following a reexposure, *Brucella abortus* was isolated at parturition from only 1 other ceased reactor. In general, the agglutination titers of the sera of the different ceased reactors were either lower or approximately the same during the second as during the first infection. These results, combined with others on this general subject obtained elsewhere, add to the general belief that an active immunity to *Brucella abortus* is engendered by an infection with virulent organisms. From the experience obtained in these experiments, it appears that the immunity thus engendered is of relatively long duration.

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## CONTENTS

	Page
Influence of Distribution of Rainfall and Temperature on Corn Yields in Western Iowa (Key No. M-2) - - - - -	533
E. E. HOUSEMAN AND F. E. DAVIS	
Resistance of Tobacco to Bacterial Wilt ( <i>Bacterium Solanacearum</i> ) (Key No. G-1269) - - - - -	547
EDWARD E. CLAYTON AND THOMAS R. SMITH	
Extent of Pathogenicity of Hybrids of <i>Tilletia Tritici</i> and <i>T. Levii</i> (Key No. G-1272) - - - - -	553
G. S. HOLTON	



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## INFLUENCE OF DISTRIBUTION OF RAINFALL AND TEMPERATURE ON CORN YIELDS IN WESTERN IOWA<sup>1</sup>

By E. E. HOUSEMAN, *junior agricultural statistician, Agricultural Marketing Service, United States Department of Agriculture, and research associate, Iowa State College of Agriculture and Mechanic Arts*, and F. E. DAVIS, *associate agricultural statistician, Agricultural Marketing Service, United States Department of Agriculture, and resident collaborator, Iowa State College of Agriculture and Mechanic Arts*<sup>2</sup>

### INTRODUCTION

Investigators who have examined the effect of weather on corn yields in Iowa have found little relation between yield and rainfall. Most studies have been made from State averages of rainfall and corn yields; however, in some cases smaller geographical areas, such as the county, have been used and the effect of monthly rainfall examined. Since the relation between yield and rainfall may vary with different soil or cultural conditions, it is thought that higher correlations might be found for still smaller areas, such as townships. There is also the possibility of getting slightly better correlations by lessening the intervals of time because the average effect of rainfall on yield may differ somewhat from one part of the month to another.

One of the primary objectives of this investigation was to study more in detail the relation between corn yield and the seasonal distribution of rainfall in Iowa for a uniform soil type, by using successive short intervals during the growing season. The finding of relationships for one soil type may lead to an inquiry as to how such relations vary with different soils and other natural or cultural conditions. The plan of study extended to the effect of temperature as well as of rainfall; also, to the possibility of pooling data from several locations in lieu of using data for a long series of years.

Not only are the relations between weather and corn yields of considerable scientific importance, but they may also be of value in forecasting annual yields of corn. There is an ever increasing demand for estimates of crop production, and this presupposes an estimate of yield. The demand comes from farmers, handlers, processors, and distributors of agricultural products, manufacturers and distributors of products to farmers, and banking institutions. Furthermore, fiscal, action, and service agencies of the Government must have accurate reports. In the past, estimates have been made for large geographical areas—the entire country, regions, or States. Now, estimates are needed for smaller areas, such as counties or townships, and for dates further in advance of harvest; the problem then becomes more than one of estimating what has been produced. It necessitates, at specified times during the growing season, an

<sup>1</sup> Received for publication February 13, 1942. This investigation is the result of research projects financed by funds appropriated by the Bankhead-Jones Act of 1935 for the U. S. Department of Agriculture "to conduct research into laws and principles underlying basic problems of agriculture in its broadest aspects."

<sup>2</sup> The study was made in cooperation with the Iowa State College of Agriculture and Mechanic Arts. The authors express their appreciation to W. G. Cochran and George W. Snedecor of Iowa State College of Agriculture and Mechanic Arts for helpful suggestions and criticisms.

appraisal of the current growing conditions jointly with crop development to that time.

It is not to be supposed that any number of studies of this nature will replace the necessity for the crop statistician. Instead, it is assumed that further information as to the importance and effect of various weather factors will help him to appraise more accurately the situations that he meets. It remains for the statistician to take the basic information and work up serviceable indications for particular areas and years somewhat in the fashion suggested by Wallace (8).<sup>3</sup> Each area and probably each year is a specific problem.

#### PREVIOUS INVESTIGATIONS

For the margins of the Corn Belt earlier investigators, among them Mattice (5), have found a number of aspects of weather (for example, July rainfall, June temperature, or accumulated degrees above 90° F.) to be related to corn yields. Hodges (4), studying weather and corn yields in Kansas, showed a high negative correlation between July temperatures and corn yields, and was able to build up a multiple curvilinear regression which gave a correlation of 0.9+ for certain areas of Kansas and the State as a whole. Using simple correlation methods, Rose (6) studied data for 55 counties scattered over the Corn Belt. At the eastern border in Ohio he observed, as had Smith (7) earlier, that July rainfall had a positive correlation, 0.4 to 0.6, with corn yields. June temperature was also positively correlated with corn yield. On the southern, southwestern, and northwestern margins, July temperatures and corn yields were negatively correlated. At the southern margin, there was a positive correlation between July rainfall and yield. No single climatic factor, however, gave statistically significant correlations with yield over all parts of the Corn Belt. At divergent points within the same States, Rose found given aspects of weather to be both positively and negatively correlated with yield.

For the heart of the Corn Belt Rose obtained no high correlations, and consistent patterns were lacking for many of the aspects of weather. Previously, Wallace (8), using similar methods, obtained about the same results and concluded that the problem of predicting corn yields from weather is relatively simple in the southern half of the Corn Belt as compared with the northern half.

As to the lack of correlation in the heart of the Corn Belt, Rose in his conclusion states, "Presumably corn yield in this core area is somewhat affected by the factors significant on the surrounding margins; but, with several factors operative—perhaps first on one, then on the other side of the optima, and thus with conditions generally favorable—variation in any one factor has little effect by itself."

A hypothetical curve representing the relationship between a weather factor and yield can be drawn. As the amount of a given aspect of weather increases, a point is reached beyond which an additional amount does not increase the yield. This point is called "optimum." In a region where near-optimum conditions prevail, a unit deviation in the weather factor has relatively little effect on yield, whereas if the weather factor varies about a point at some distance to either side of the optimum, there is a comparatively large change in yield for each unit change in the weather factor. If the given aspect

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 545.

of weather fluctuates about a point at or near optimum, both a larger or smaller amount of the weather factor may have a diminishing effect on yield. An assumption of linearity is most nearly valid in regions where the aspect of weather always remains above or below the optimum.

With a multitude of factors affecting yield, all of which are generally not far from optimum, as is the case in Iowa, it appears unlikely that there is much correlation between yield and a given aspect of weather, such as July rainfall. At the margins of the Corn Belt the situation differs in that one or two weather factors generally remain at an appreciable distance from optimum, and hence play an important part in determining yield.

#### SELECTION OF DATA

As previously stated, one of the objectives is to study further the relation between yield and rainfall (and temperature) under uniform soil and cultural conditions, by using successive short intervals of time. R. A. Fisher (2) developed a method employing rainfall for small intervals of time. His approach, to be briefly described later, will be that of the present study.

From the annual Farm Census in Iowa there are available annual yields of corn by townships dating back to 1924, and in or near a number of townships are weather stations affording suitable weather data. For the rather complicated analysis used by Fisher a longer series than 15 or 16 years is desirable. Therefore, we examine the possibility of choosing several stations<sup>4</sup> and pooling the data in order to increase the number of station years. Although such a procedure is not always successful, it can be subjected to available tests.

In western Iowa it was possible to select townships from an extensive area which were alike with respect to: (1) Soil area—Missouri loess; (2) soil type—Marshall silt loam; (3) slope of land—10 percent or less; (4) percentage of land in intertilled crops—40 to 50 percent; (5) depth of soil—4 inches or over.

A wide distribution of townships was sought in order that weather might differ as much as possible in each year. Six townships and weather stations, two in each of the three western crop-reporting districts, were chosen:

Weather station:	Township
Rock Rapids.....	Rock.
Alton.....	Nassau.
Carroll.....	Maple River.
Little Sioux.....	Little Sioux.
Atlantic.....	Grove.
Corning.....	Jasper.

#### RAINFALL ANALYSIS

In Iowa early spring rains are considered beneficial to the corn crop because they build up the moisture reserve in the subsoil. From planting time through early cultivation (for 5 weeks beginning approximately May 5) rainfall is adequate in most years and possibly greater than optimum, for at that time excess rainfall might impede cultivation or be accompanied by temperatures which are too low for proper germination and development of the seedlings. From early

<sup>4</sup> Station refers to both the weather station and the associated township.

July to late August more than normal rainfall is generally thought to be favorable to corn growth. The greatest benefits probably accrue in late July or the first part of August when the normal rainfall is at a low level, and the transpiration surface of the plant has reached a maximum.

In view of these facts one can conceive a graph which would picture the average effect on corn yield of an inch of rain at any time between May 1 and October 1. This graph can be thought of as a smooth curve which changes rather slowly since the effect of an inch of rain would not be appreciably different if it came a day or two earlier or later. Roughly, one might expect such a curve to start with a moderate positive value, recede to a minimum around June 1, rise to a maximum near August 1, and then decrease until the end of the season. It is clear that the curve can be represented by a mathematical function of time, but how can the function be evaluated?

First, we must specify the function. For our problem a third degree equation is suitable. This may be written

$$(1) \quad a = \alpha_0 \xi'_0 + \alpha_1 \xi'_1 + \alpha_2 \xi'_2 + \alpha_3 \xi'_3$$

where  $a$  represents the effect of an inch of rain, the  $\alpha$ 's are constants to be determined, and  $\xi'_0$ ,  $\xi'_1$ ,  $\xi'_2$ , and  $\xi'_3$  are orthogonal polynomials (3) (in this case orthogonal functions of time) of degrees 0, 1, 2, and 3 respectively. We shall call equation (1) the regression equation and the  $\alpha$ 's the regression coefficients.

The evaluation of the regression coefficients involves tabulation of the rainfall data by short intervals of time; in this study thirty-one 5-day intervals were used. From the rainfall data a set of quantities,  $\rho_0$ ,  $\rho_1$ ,  $\rho_2$ , and  $\rho_3$ , is computed for each season. These quantities are coefficients of polynomials of the form

$$(2) \quad \rho_0 \frac{\xi'_0}{S(\xi'^2_0)} + \rho_1 \frac{\xi'_1}{S(\xi'^2_1)} + \rho_2 \frac{\xi'_2}{S(\xi'^2_2)} + \rho_3 \frac{\xi'_3}{S(\xi'^2_3)}$$

fitted to the rainfall data for each season. Then, the  $\alpha$ 's are computed by the usual rules of multiple regression. The theory of this device is presented by Fisher (2). Equations (1) and (2) are taken from Davis and Pallesen (1).

The records available for this investigation afforded data for only 15 years, 1924-38. Of these 15 years, 1934 and 1936 were abnormal, yields in many townships being 5 bushels per acre or less. The poor yields were caused by long periods of very high temperature together with the lack of rainfall. This introduces some complications since the inclusion of these 2 years in the analysis could lead to statistically significant results, whereas if they were omitted different conclusions might be drawn about the influence of temperature and rainfall on yield. Thus it was thought advisable to make the analysis with all years included and then with the years 1934 and 1936 omitted.

To develop a regression for predicting differences in yield from year to year, one should use only the yearly means as proximately relevant data. But, in our case more data are desired, and it is thought that a better estimate of the regressions might be obtained by pooling the experiences at the six stations. By means of the arithmetic procedure known as the analysis of variance, the total variability in the combined data was separated into two parts; namely, the variability among



station means, and the intrastation variation, which is an average of the variability arising at each of the six stations. The first component was influenced not only by differences in weather conditions but also by variations in farm practices, average soil fertility, and other factors that could not be taken into account in selecting the townships; hence, variation among station means was excluded and the regression analysis based on the intrastation variation.

With 1934 and 1936 omitted the regression coefficients together with their standard errors are:

$$\begin{aligned}\alpha_0 &= 0.1194 \pm 0.1828 & \alpha_2 &= -0.0006108 \pm 0.002431 \\ \alpha_1 &= 0.005113 \pm 0.01819 & \alpha_3 &= -0.0006270 \pm 0.0003151\end{aligned}$$

None of the  $\alpha$ 's is statistically significant except the coefficient of the cubic term,  $\alpha_3$ , which is just at the 5-percent level. This would indicate that in general a little more rainfall than average following the middle of the season and a little less before the middle has been beneficial.

The mean effect (fig. 1) of an inch of rain more than average for any 5-day period during the season is obtained from the equation

$$a = 0.1194\xi_0' + 0.005113\xi_1' - 0.0006108\xi_2' - 0.0006270\xi_3'$$

by substituting the values of the  $\xi$ 's for  $n=31$ . In figure 1 the  $x$ 's mark the standard error of the ordinates of the curve at four different points. The large standard errors show that the average effect of rainfall on corn yield is inaccurately determined. None of the points on the curve is at a distance of more than two standard deviations from the 0 line. The nonsignificance of the regression curve is confirmed by the analysis of variance (table 1), since the value of the  $F$  ratio is only 1.4. The regression appears to be of little or no aid in estimating the yield of corn.

When 1934 and 1936 are included a statistically significant regression is obtained, the regression accounting for 29 percent of the total variation in yield. The total sum of squares, however, was increased from 3,024 to 12,224, and even though the regression was significant, the mean square of the deviations from regression was 109.0—a much larger quantity than either the corresponding mean square of 41.0 (table 1) or 42.0, the variance of the actual yields for the 13 more common years.

TABLE 1.—*Rainfall regression analysis based on the intrastation variation, 1924-38 (1934 and 1936 omitted)*

Source of variation	Degrees of freedom	Sum of squares	Mean square	$F$
Regression .....	4	232	58.0	1.4
Deviations from regression .....	68	2,792	41.0	
Total .....	72	3,024	42.0	

The intrastation variation is actually a weighted average of the variation among yearly means and the station by year interaction; i. e., the variation at the stations after allowing for differences in the yearly means and differences in the station means. Thus, the regression based on the intrastation variation is a weighted mean of two

regressions, one based on the variation among years and the other on the station by year interaction. Ordinarily, to predict difference from year to year one would use the regression among years. In case the regression among years and the regression based on interaction differ significantly, the intrastation regression would not be appropriate for estimating differences in yield from year to year. It will be pointed out later that the mean square of the deviations from the intrastation regression (41.0 in table 1) is very likely to give a negatively biased estimate of the error mean square for estimating

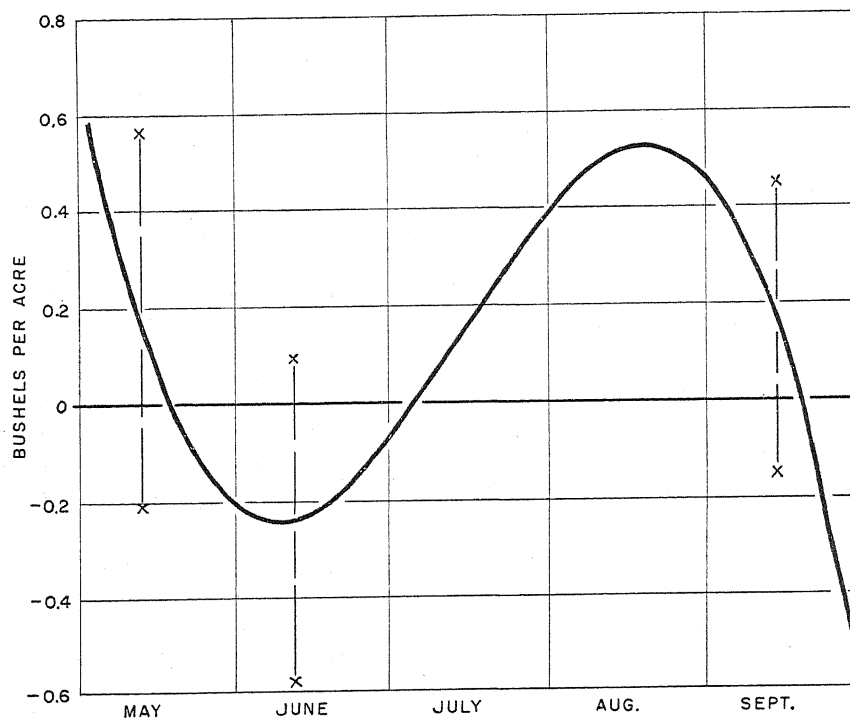


FIGURE 1.—Mean effect of an inch of rainfall above average on the yield of corn in western Iowa.

yearly differences. These points will be examined in the temperature analysis. For the case of rainfall such a study is probably not worth while.

#### TEMPERATURE ANALYSIS

In the area under investigation it is believed that high temperature frequently has a harmful effect on the yield of corn. To discover the effect of high temperature, maximum daily temperature has been used rather than the mean or minimum. The temperature data were handled in the same manner as those of rainfall. In place of total rainfall for 5-day periods we now have 5-day averages of daily maximum temperatures.

When the regression is based on intrastation variation including 1934 and 1936, the coefficients,  $\alpha$ 's, and their standard errors are:

$$\begin{aligned}\alpha_0 &= -0.04472 \pm 0.01103 & \alpha_2 &= 0.001768 \pm 0.003806 \\ \alpha_1 &= 0.004060 \pm 0.001591 & \alpha_3 &= 0.001823 \pm 0.003713\end{aligned}$$

All the coefficients are statistically significant.

The regression curve (fig. 2) is the graph of

$$a = -0.04472\xi'_0 + 0.004060\xi'_1 + 0.001768\xi'_2 + 0.001823\xi'_3$$

As indicated by the rather small standard deviations of the ordinates, the curve is fairly well determined (fig. 2). From the third week

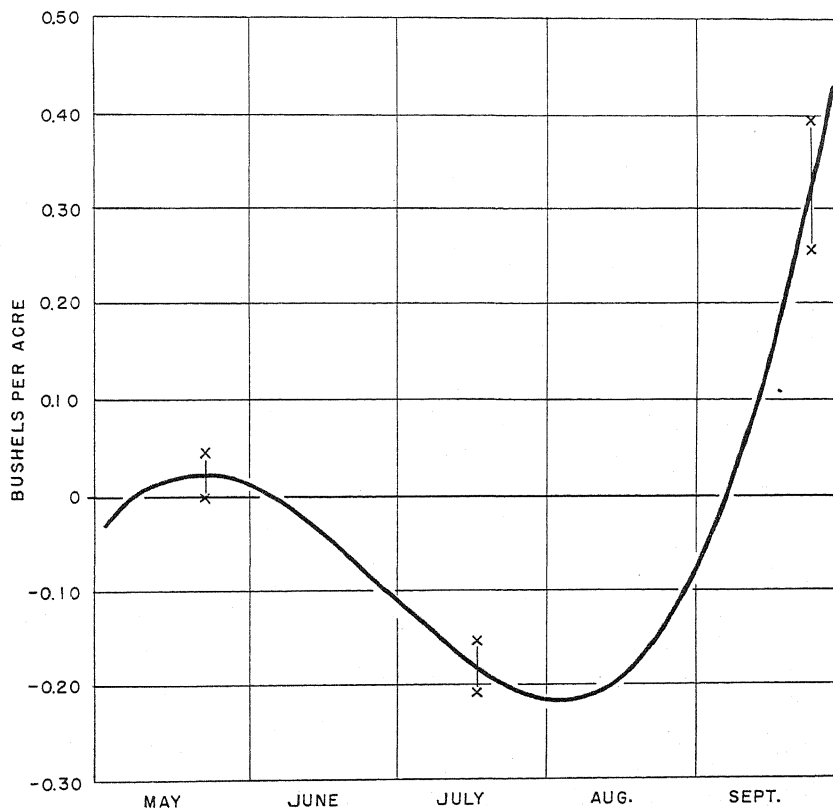


FIGURE 2.—Mean effect of a degree of maximum temperature above average on the yield of corn in western Iowa.

of June to the first of September the curve shows that more than average<sup>5</sup> maximum temperature decreases the yield of corn. Before the middle of June the effect is relatively small. High temperatures during the latter part of September are evidently beneficial to corn. The effect, however, as shown by the curve is probably exaggerated. Perhaps a better picture could be obtained by extending the ending date about 2 weeks and fitting a fourth degree polynomial. One would then expect the curve to reach a maximum some time around October 1.

<sup>5</sup> The average is established on the basis of the 15 years.

From the analysis of variance (table 2) we see that the regression is highly significant, and accounts for 58 percent of the total variation. The situation here is similar to that for rainfall. When 1934 and 1936 are omitted the *F* ratio drops from 27.7 to 3.8 (table 3), but the regression is still highly significant. Hence in more normal years the existing variations in temperatures in western Iowa usually exert a greater effect on corn yield than the existing fluctuations in rainfall.

TABLE 2.—Temperature regression analysis based on intrastation variation, 1924–38 (1934 and 1936 included)

Source of variation	Degrees of freedom	Sum of squares	Mean square	<i>F.</i>
Regression.....	4	7,100	1,775**	27.7
Deviations from regression.....	80	5,124	64.0	
Total.....	84	12,224		

\*\*Highly significant.

TABLE 3.—Temperature regression analysis 1924–38 (1934 and 1936 omitted)

Source of variation	Degrees of freedom	Sum of squares	Mean square	<i>F.</i>
Regression.....	4	556	139**	3.8
Deviations from regression.....	68	2,468	36.3	
Total.....	72	3,024		

\*\*Highly significant.

The regression curve with 1934 and 1936 omitted (fig. 3) shifts upward and to the right, shortening the period of time for which more-than-average maximum temperature tends to decrease the yield. It should be remembered that in this case the average temperatures during the season are generally a few degrees lower since 2 years with extremely high temperatures are excluded.

At this point let us examine the appropriateness of the regression based on intrastation variation for estimating differences in yield from year to year.<sup>6</sup> To learn about this, the intrastation differences were analyzed into two parts, differences among years and the station by year interaction. A regression based on each part was computed. The  $\alpha$ 's for the regression among years are:

$$\begin{aligned}\alpha_0 &= -0.03746 \pm 0.2675 & \alpha_2 &= 0.002028 \pm 0.0^39951 \\ \alpha_1 &= 0.004752 \pm 0.003795 & \alpha_3 &= 0.0^31846 \pm 0.0^48712\end{aligned}$$

whereas those for the regression based on interaction are:

$$\begin{aligned}\alpha_0 &= -0.09106 \pm 0.02294 & \alpha_2 &= 0.01039 \pm 0.0^34850 \\ \alpha_1 &= 0.002553 \pm 0.004130 & \alpha_3 &= 0.0^31831 \pm 0.0^48709\end{aligned}$$

Differences between the various pairs of corresponding coefficients were tested, but none was found to be statistically significant. Hence the two regressions seem to be in close enough agreement to justify

<sup>6</sup> The discussion from here on refers to the case with 1934 and 1936 included.



the procedure of pooling the data and using the average regression (based on the intrastation variation), which supposedly gives a better estimate of the true regression coefficients. Agreement between the two curves is shown in figure 4.

The analyses of variance for the regression among years and the regression based on interaction are given in tables 4 and 5 respectively. It is of importance to note that even though the average regression may be legitimate, the error mean square from table 2 underestimates the error variance for estimating yearly differences because of the intrayear correlation between the station yields. In table 4 the sums of squares are six times those that would be obtained if one actually used the 15 yearly means; hence, the standard error associated with

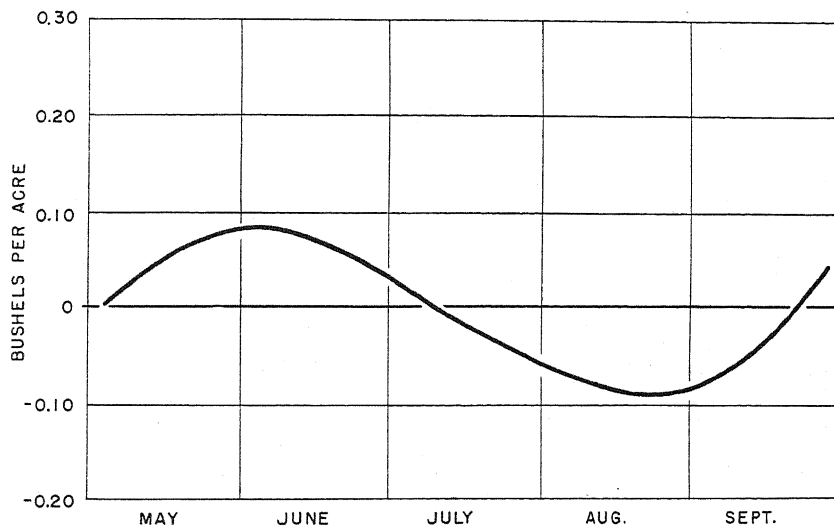


FIGURE 3.—Average effect of maximum temperature—1934 and 1936 omitted.

an estimate of the yearly mean yields for the six stations using the regression among years is  $\sqrt{321/6}=7.3$  bushels. If one uses the average regression to estimate differences among the same 15 yearly means, a standard error of 6.7 is obtained. This is considerably higher than the incorrect estimate,  $\sqrt{64.0/6}=3.3$ , resulting from the use of the error mean square in table 2.

TABLE 4.—Temperature regression analysis based on variation among years, 1924-38

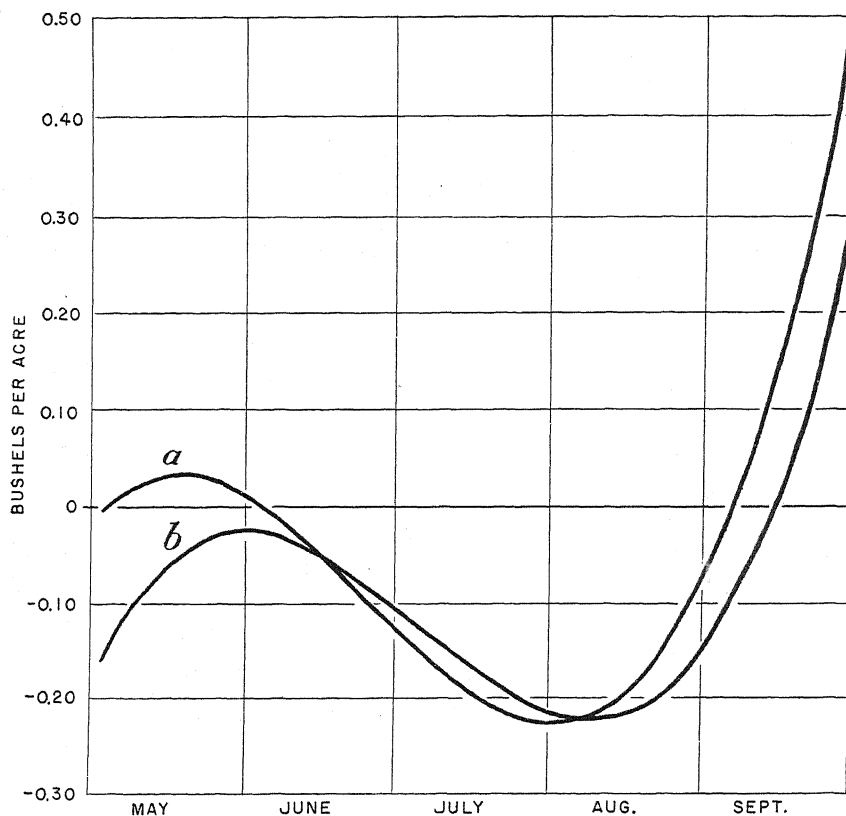
Source of variation	Degrees of freedom	Sum of squares	Mean square	F.
Regression .....	4	6,315	1,579*	4.9
Deviations from regression .....	10	3,207	321	
Total .....	14	9,522		

\*Significant.

TABLE 5.—Temperature regression analysis based on interaction, 1924-38

Source of variation	Degrees of freedom	Sum of squares	Mean square	F.
Regression .....	4	949	237**	8.9
Deviations from regression .....	66	1,753	26.6	
Total .....	70	2,702		

\*\*Highly significant.

FIGURE 4.—Curves corresponding to the regression among years *a*, and the regression based on interaction (*b*).

The regression curve in figure 2 is an average of the six curves for the individual stations. If these six curves should differ by a considerable amount, one could do better from the prediction point of view by using the appropriate station curve. To check on this point, a separate analysis was computed for each of the six stations (table 6). From table 7 we note that the mean square for differences among the regressions, 33.1, is small in comparison to 74.3, the average mean square for deviations from individual regressions. Hence, from the prediction point of view, there is probably nothing to be gained by using individual regressions (fig. 5) instead of the average regression.

The six curves are much alike, and there is no apparent tendency for them to be arrayed either according to the average yield for each station or by location of the station.

Suppose there were two neighboring townships, A with a high mean yield and B with a low mean yield. For a given date one might expect the magnitude of the effect of temperature on yield to be greater for township A than township B; i. e., as the mean yield increases, the regression curve may become more pronounced. If the

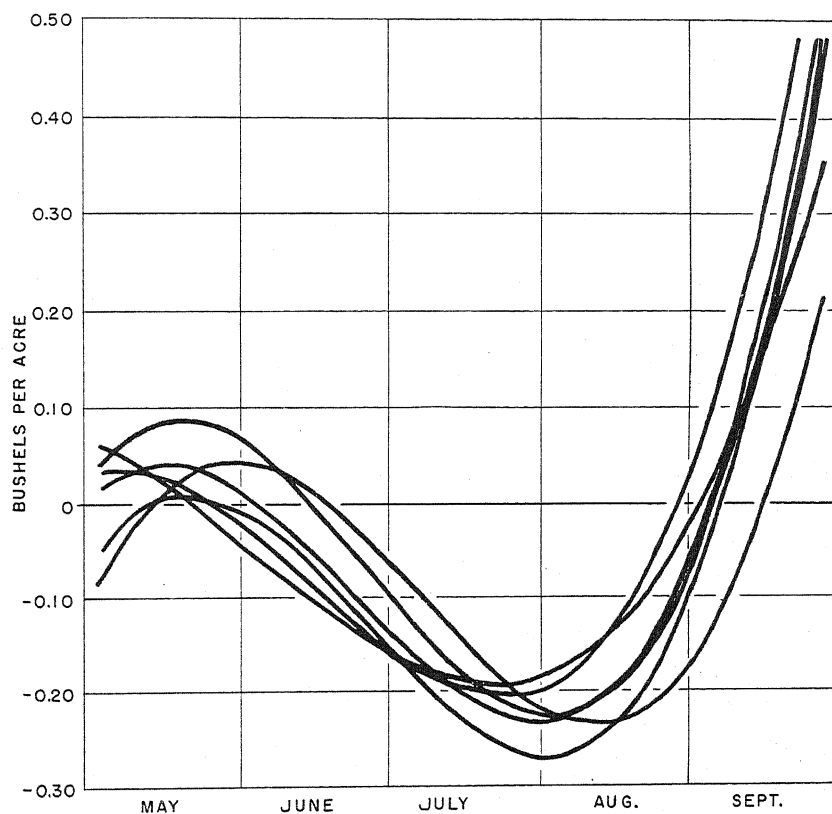


FIGURE 5.—Maximum temperature curves for the individual stations.

effect of maximum temperature at any given date is approximately proportional to the mean yield, one could obtain a single regression curve, stating the effect as a percentage of the average yield, that would be applicable to different areas which have about the same average weather conditions even though the mean yields of these areas differ. The average yields at the six stations, in the same order as in table 6, for the 15 years studied are 31.3, 33.8, 38.3, 34.0, 34.9, and 32.9. The range of the means is rather small for purposes of trying out the above idea. Since only a very small amount of additional clerical work was needed, such a regression was computed for the six stations. In this case the  $F$  ratio was 29.7 as compared with 27.7 in table 2.

TABLE 6.—*Temperature regression analysis for each station, 1924-38*

Station	Mean square		
	Regression (4 degrees of freedom)	Deviation from regression (10 degrees of freedom)	F <sup>1</sup>
Rock Rapids.....	208	61.3	3.4
Alton.....	338	58.3	5.8
Carroll.....	303	92.5	3.3
Little Sioux.....	257	85.0	3.0
Atlantic.....	428	57.4	7.4
Corning.....	406	91.5	4.4

<sup>1</sup> F at the 5-percent level for degrees of freedom 4 and 10 is 3.5.

TABLE 7.—*Differences among individual station regressions*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Deviations from average regression within stations.....	80	5,124	64.0
Deviations from individual station regressions.....	60	4,461	74.3
Difference between regressions.....	20	663	33.1

### SUMMARY AND CONCLUSIONS

By using the method developed by Fisher (2), corn yield in western Iowa was related to rainfall and maximum temperature. Practically none of the variation in corn yield was accounted for by variation in the amount and distribution of rainfall. Yield was more closely related to temperature than to rainfall. The standard deviation of yield from year to year was 10.6, as compared with the standard error of estimate 6.7, when using the temperature regression.

At the outset of this study the question of how the effect of rainfall on corn yield might differ from one soil type (or cultural condition) to another was raised. Since the correlation between rainfall and yield is small, such a study would appear unprofitable. Rainfall in western Iowa is usually near optimum except for part of the time when temperature is high.

In lieu of data for a long series of years at a single location, data from six locations were pooled. This procedure gave a suitable estimate of the temperature-regression equation, but produced an underestimate of the error for estimating differences from year to year. The reason for the underestimate of error was the intrayear correlation between the station yields.



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# RESISTANCE OF TOBACCO TO BACTERIAL WILT (*BACTERIUM SOLANACEARUM*)<sup>1</sup>

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## INTRODUCTION

The bacterial wilt or Granville wilt (*Bacterium solanacearum* E. F. Smith, syn. *Phytophthora solanacearum* (E. F. Smith) Bergey et al.) is a common and serious disease of flue-cured tobacco (*Nicotiana tabacum* L.). It is extremely destructive in north-central North Carolina, where, on about 75,000 acres of sandy soil (mostly of the Granville type) is produced tobacco of exceptionally fine body, aroma, and color. Bacterial wilt has materially reduced the value of this land. For example, land free of wilt in this area would find ready sale at \$100 an acre, while the price of badly contaminated land is now about \$25 an acre. This land will grow many other crops, but because of the low fertility level, it is not profitable to grow these crops in competition with other areas. Sandy loam types of soil of the Granville, Durham, Norfolk, and Marlboro series are most seriously affected by wilt.

The wilt disease has now been known for about 60 years, but despite careful investigations in this country from 1903 to 1917 and similar studies in the Netherlands East Indies and Japan over a much longer period, no adequate method of control has been developed, and growers in seriously affected areas regularly lose 20 percent or more of their tobacco crop from wilt.

Breeding for resistance was one of the first methods of control suggested. In 1903, Stevens and Sackett (6)<sup>2</sup> said: "The greatest hope for the redemption of land now affected lies in the development of a variety of tobacco that can resist the disease." However, these investigators concluded their work without finding such a tobacco. In 1917, Garner et al. (1), reporting on wilt-control investigations, said that, after testing all available foreign and domestic varieties, none was found that possessed sufficient resistance to be worth while. In view of these findings active breeding work was discontinued.

In the Netherlands East Indies, work on wilt resistance has been reported by various investigators, including Honing (2), Vriend (7), Palm (5), Kuijper (4), and Koenig and Rave (3). It was early observed that the Sumatra variety of tobacco was at least as resistant as any and more resistant than most varieties. Hence, various workers have sought to increase this resistance by selection. However, this work has not met with much success; the degree of resistance so far secured is not sufficient to give protection under severe disease conditions.

Work by the Bureau of Plant Industry, United States Department of Agriculture, in cooperation with the State of North Carolina, was

<sup>1</sup> Received for publication April 15, 1942. Cooperative investigations of the Division of Tobacco Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the North Carolina Agricultural Experiment Station, and the North Carolina Department of Agriculture.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 554.

resumed in 1934. It seemed probable that available gene material had been rather thoroughly studied; therefore the first step in the investigations here reported was to secure new and more extensive collections. The chief emphasis was placed on *Nicotiana tabacum*, although some attention was given to other species of *Nicotiana*.<sup>3</sup> Many new collections of this species were obtained by special collectors<sup>4</sup> in Mexico and in Central and South America.

#### METHODS OF TESTING

Preliminary work was conducted in the greenhouse. Plants were grown and transplanted individually into 4-inch pots—10 plants per lot. When the plants were growing rapidly, holes were punched into the soil with a sharp stick to break some of the roots. These holes were then filled with inoculum consisting of broth cultures of the bacteria, diluted about 10 to 1. Critical tests could be conducted only during the warmer parts of the year, from June to October, inclusive. During the years 1934-38, 1,034 collections were tested in the greenhouse, some two or three times, and single-plant selections were made from all of the more promising strains. After greenhouse elimination the surviving strains were tested in the field at Creedmoor, N. C., on land so heavily infected that all susceptible tobacco was usually dead by midseason (fig. 1, A). Except for the wilt, however, the land used was capable of producing excellent crops of high-quality tobacco (fig. 1, B). A total of 129 introductions were subjected to careful field test during the period 1935-41. The more promising strains have been tested many times, under widely varying conditions. It has not been deemed advisable to draw definite conclusions on any genotype with less than 3 years of experimentation, as a single year's results on wilt resistance have sometimes proved very misleading.

#### RESULTS

##### RESISTANCE OF VARIOUS SPECIES OF NICOTIANA TO BACTERIAL WILT

The following species of tobacco have been tested and found highly susceptible: *Nicotiana acuminata* (Grah.) Hook., *N. alata* Link and Otto, *N. attenuata* S. Wats., *N. caesia* Suksd., *N. cavanillesii* Dun., *N. debneyi* Domin, *N. exigua* H.-M. Wheeler, *N. glauca* Grah., *N. glutinosa* L., *N. goodspeedii* H.-M. Wheeler, *N. gossei* Domin, *N. langsdorffii* Weinm., *N. longiflora* Cav., *N. maritima* H.-M. Wheeler, *N. megalosiphon* Heurck and Muell.-Arg., *N. miersii* Remy, *N. nesophila* Johnst., *N. nudicaulis* S. Wats., *N. paniculata* L., *N. plumbaginifolia* Viv., *N. quadrivalvis* Prush,<sup>5</sup> *N. raimondii* Macbride, *N. repanda* Lehm., *N. rotundifolia* Lindl., *N. rustica* L., *N. stocktoni* Brandeg., *N. sylvestris* Speg., *N. tomentosa* Ruiz and Pav., and *N. trigonophylla* Dun. All these species were at least as susceptible as ordinary varieties of *N. tabacum*, so there has appeared little reason to look for resistance to bacterial wilt among the wild relatives of cultivated tobacco.

<sup>3</sup> These were largely obtained from Dr. T. H. Goodspeed, of the University of California.

<sup>4</sup> Dr. W. A. Archer and R. Stadelman, of the Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

<sup>5</sup> This material was received under the name *N. bigelovii*. The name *quadrivalvis* antedates *bigelovii* and was used originally to describe one form of this species; hence under present rules of nomenclature it must be applied to the species as a whole.





FIGURE 1.—A, Tobacco crop, planted on land heavily infected with bacterial wilt, completely destroyed; B, a nearby crop, planted and fertilized in the same manner as that in A, shows the growth of tobacco that could have been produced on the land shown in A had it not been for the wilt.

## RESISTANCE OF NICOTIANA TABACUM TO BACTERIAL WILT

With over 1,000 new seed collections available, the writer began work in 1934 and soon found various strains that were more or less resistant. The experience of previous investigators that strains may appear quite resistant one year and later, under more severe conditions, go down before the disease almost completely, was repeatedly duplicated. However, by 1938 the available material had been quite well covered, and the truly resistant strains began to stand out clearly. By 1940, constant elimination had reduced the resistant strains to a very compact group; the susceptibility of these different genotypes to wilt is shown by table 1.

TABLE 1.—Resistance of various strains of tobacco to bacterial wilt, Creedmoor, N. C., 1940

Strain	Amount of disease <sup>1</sup>								Mean <sup>3</sup>
	Block <sup>2</sup> -								
	1	2	3	4	5	6	7	8	
Cash (susceptible control) . . . . .	100	95	100	90	87.5	97.5	90	82.5	92.8
Davis Special . . . . .	92.5	92.5	90	95	57.5	60.5	57.5	72.5	77.2
T. I. 79A . . . . .	40	50	32.5	55	35	22.5	20	30	35.6
Xanthi . . . . .	30	37.5	27.5	20	25	15	20	15	23.8
79-X <sup>5</sup> . . . . .	20	25	25	10	32.5	5	5	5	15.9
T. I. 448A . . . . .	7.5	15	12.5	12.5	12.5	12.5	10	27.5	13.8

<sup>1</sup> 100=maximum of disease (all plants dead); 0=no disease.

<sup>2</sup> Blocks 1 to 4, after tobacco; infection very heavy. Blocks 5 to 8, after corn; infection moderately heavy.

<sup>3</sup> Significant difference between means at 5-percent level, 9.4; at 1-percent level, 12.5.

<sup>4</sup> Approximately 50 plants per plot.

<sup>5</sup> F<sub>5</sub> from a cross of T. I. 79A and Xanthi.

Table 1 brings out important points in connection with resistance to bacterial wilt. In blocks 5 to 8, Davis Special was distinctly more resistant than the Cash control. These plots were on moderately infected land, since it had been cropped to corn the year previous. In blocks 1 to 4, Davis Special was no more resistant than the Cash control. These plots were on heavily infected land that had produced a diseased crop of tobacco the previous year. It has been a common observation that slightly wilt-resistant genotypes, such as Davis Special, show up to advantage under less severe wilt conditions. For example, they may appear quite resistant early in the summer and later be just as badly affected by wilt as ordinary susceptible tobacco. T. I. 79A and Turkish Xanthi have both displayed considerable wilt resistance. As table 1 shows, they were consistently much superior to Davis Special. A cross between these two yielded 79-X, which was more highly resistant than either of the parent strains. T. I. 448A was another highly resistant strain and possessed other very desirable characters, as will be noted later. The ability of these two strains to grow normally on wilt-infected land is illustrated in figure 2.

In table 2, data are given for four separate single-plant selections of T. I. 448A and 79-X.

As the complete destruction of the Gold Dollar control in 1941 indicates, wilt was very severe that year. Under these conditions both Xanthi and T. I. 79A were severely affected. On the other hand, T. I. 448A and 79-X strains show no more wilt than in 1940. Approximately 92 percent of the plants of both resistant strains were alive and



FIGURE 2.—Tobacco growing on the heavily wilt-infected land shown in figure 1, A. The row of ordinary susceptible tobacco (Jamaica) in the center has been completely destroyed. T. I. 448A (left) and a T. I. 79A-Xanthi hybrid (right), two highly wilt-resistant strains, are growing normally.

growing vigorously on September 12, 1941, and essentially the same results were obtained in 1939 and 1940. It is questionable whether significant differences exist between the different selections. These and similar data suggest that both T. I. 448A and 79-X were essentially homozygous for resistance. Both these genotypes have been thoroughly tested over a 3-year period without any indication of a break-down in their resistance, even during the very severe wilt year of 1939. A summary of the pertinent data regarding the resistant strains follows.

TABLE 2.—Resistance of various strains of tobacco to bacterial wilt, Creedmoor, N. C., 1941

Strain	Amount of disease <sup>1</sup>				
	Block—				Mean <sup>2</sup>
	1	2	3	4	
T. I. 448A-1.....	6	10	18	20	13.5
T. I. 448A-2.....	12	6	15	25	14.5
T. I. 448A-3.....	19	20	13	32	21.0
T. I. 448A-4.....	13	1	5	6	6.2
79-X-1.....	23	13	28	13	19.2
79-X-2.....	8	10	14	18	12.5
79-X-3.....	7	2	16	16	10.2
79-X-4.....	10	16	10	13	12.2
Xanthi.....	28	40	65	54	46.8
T. I. 79A.....	64	78	84	55	70.2
Gold Dollar (susceptible control).....	100	100	100	100	100.0

<sup>1</sup> 100=maximum of disease (all plants dead); 0=no disease.

<sup>2</sup> Significant difference between means at 5-percent level, 11.9; at 1-percent level, 16.1.

<sup>3</sup> Approximately 45 plants per plot.

#### STRAINS HAVING SLIGHT WILT RESISTANCE

Strains having slight wilt resistance are typified by Davis Special (table 1) and a considerable number of foreign collections. They often showed quite striking evidences of resistance under mild conditions, but under severe conditions appeared as completely susceptible



Certain of these genotypes, having good flue-cured quality, give indications of value for crossing with the more highly wilt-resistant strains. They have no value for crossing with susceptible genotypes, as the resistance has proved both inadequate and impossible to recover in full.

#### STRAINS HAVING MODERATE WILT RESISTANCE

The best strains having moderate wilt resistance were T. I. 79A and Turkish Xanthi. Most Turkish varieties were not wilt-resistant. Both T. I. 79A and Turkish Xanthi had very small leaves, and the great majority of the selections from crosses made between these and flue-cured varieties also had small leaves. In fact, the general type of these progenies has been uniformly poor, and in addition it has proved very difficult to recover full resistance after a cross to susceptible tobacco. The only really valuable result from the work with these strains has been strain 79-X (tables 1 and 2), which was selected out of the cross T. I. 79A  $\times$  Xanthi.

#### STRAINS HAVING HIGH WILT RESISTANCE

There are two strains having high wilt resistance, namely, 79-X, just referred to, and T. I. 448-A. Both have shown less than 10 percent of plants actually killed by wilt over a 3-year period of testing under disease conditions so severe that ordinary tobacco was 100 percent destroyed.

79-X is a poor plant type, but a study of both genetic behavior and pathological reactions indicates that the resistances of 79-X and T. I. 448A are basically different and are probably controlled in major part by different genes. Consequently, just as the highly wilt-resistant 79-X was obtained by crossing two moderately resistant strains that had different types of resistance, so it seems possible that a completely wilt-immune genotype may be obtained from a crossing of 79-X and T. I. 448A. This would be a useful strain to have in reserve.

Discussion of T. I. 448A has been left until last, as of all the 1,034 collections tested, this is the only one that seems likely to provide a practical solution to the problem of bacterial wilt control. T. I. 448 was obtained in Tolima, Colombia, and was labeled by the collector as a mixture of Castillo negro, Castillo blanco, and Pina. From greenhouse plantings of T. I. 448 a single plant was selected that appeared highly resistant to both bacterial wilt and mosaic. A plant of the T. I. 448A strain is shown in figure 3, A, and a plant of Gold Dollar, a flue-cured type, in figure 3, B. Plant measurements of these two are given in table 3.

The differences in general plant shape are clearly shown in figure 3. The data in table 3 indicate that T. I. 448A has more and shorter leaves than flue-cured tobacco. However, T. I. 448A did cure-out to a fair quality and proved neutral with respect to aroma. Of greater importance is the fact that numerous  $F_3$  lines from T. I. 448A  $\times$  flue-cured, grown in the field in 1941, gave a cured leaf that strongly resembled flue-cured tobacco, and some possessed the full resistance of the T. I. 448A parent. Hence, while it is not the purpose of this paper to report on the breeding work, it can be noted that satisfactory wilt-resistant flue-cured tobaccos are now definitely in prospect.



TABLE 3.—Plant measurements of T. I. 448A and flue-cured tobacco

Measurement	T. I. 448A				Flue-cured			
	Plant—			Mean	Plant—			Mean
	1	2	3		1	2	3	
Height.....inches	60	60	60	60	64	60	66	63.3
Leaves.....number	28	26	27	27	23	23	23	23
Leaf length <sup>1</sup> .....inches	21	20.3	21.7	21	22.3	23	26	23.8
Leaf breadth.....do.	11	10.3	11.7	11	12.7	11.5	13.7	12.6

<sup>1</sup> Maximum.

A very interesting aspect of the resistance of T. I. 448A to wilt concerns the early- and late-season behavior of this type. Early in the summer a considerable percentage of the young plants often showed wilt symptoms. Less than 10 percent died of the wilt, and practically all the deaths occurred in this early period. T. I. 448A plants showing early-season symptoms were labeled and observed throughout the summer. The great majority recovered and grew normally, with the result that during midsummer and late summer wilt symptoms were difficult to find. On the other hand, with such types as T. I. 79A, many large plants approaching maturity often wilted and died.



FIGURE 3.—A, T. I. 448A, a highly wilt-resistant strain that grows vigorously and has leaves of good size; B, Gold Dollar, a typical flue-cured variety. (Note the conical shape of plant and the "stand-up" type of leaf growth.)

## SUMMARY

Search for resistance of tobacco (*Nicotiana tabacum*) to bacterial wilt (*Bacterium solanacearum*) was begun in 1934.

None of the wild *Nicotiana* species tested showed resistance.

Of 1,034 collections of *Nicotiana tabacum*, obtained chiefly in Mexico and in Central and South America, a very few showed resistance.

By crossing T. I. 79A and Turkish Xanthi, two moderately resistant strains, a highly resistant genotype, 79-X, was developed, but it was a very poor type of tobacco.

A highly wilt-resistant collection of good type, T. I. 448A, was obtained from Colombia. Over a 3-year period this strain always showed less than 10 percent mortality, even though disease conditions were so severe that susceptible tobacco was 100 percent destroyed.

T. I. 448A was also highly resistant to common tobacco mosaic.

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# EXTENT OF PATHOGENICITY OF HYBRIDS OF *TILLETIA TRITICI* AND *T. LEVIS*<sup>1</sup>

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## INTRODUCTION

The demonstration of heterothallism and hybridization in *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn by Flor (2)<sup>2</sup> in the United States was confirmed by Hanna (3) in Canada and Becker (1) in Germany. Each of these workers presented evidence of the heritability of chlamydospore morphology, the smooth spore character of *T. levis* apparently being dominant over the reticulate character of *T. tritici*. Furthermore, Becker (1) obtained some evidence of the inheritance of pathogenicity in crosses between two races of *T. tritici*. In his studies of two hybrids the inheritance of pathogenicity was found to be intermediate in one and recessive in the other, as indicated by the reaction of the highly susceptible variety, Panzer. In 1938 the writer (4) presented evidence of the inheritance of chlamydospore characteristics and pathogenicity in a cross between *T. tritici* and *T. levis*. A pathogenically distinct race of *T. tritici*, derived from this cross, had the pathogenic factors of both parents, and it was observed that the reticulate character was dominant. In a more recent report (6) evidence was presented to show that pathogenicity factors may be inherited transgressively. The purpose of this paper is to present the results of extensive studies on the pathogenicity of species and race hybrids through several generations. No consideration will be given to the inheritance of chlamydospore characteristics.

## MATERIAL AND METHODS

Races T-8, T-9, T-10, and T-12 of *Tilletia tritici* and L-7 and L-8 of *T. levis* were used in these investigations. These races are designated by the numbers assigned to them by Rodenhiser and Holton (7). All cultures used for inoculation purposes were of monosporidial origin and were obtained by isolating single primary sporidia from the promycelia of germinating chlamydospores. Inoculations with cultures were made by the method described by the writer in 1938 (5), while inoculations with chlamydospores were made by dusting the seed at the rate of 0.5 gm. of spores to 100 gm. of seed. The hybrids are designated by number and are pedigreed as to race, chlamydospore, and sporidium.

Hindi (C. I.<sup>3</sup> 8454), a highly susceptible variety of spring wheat (*Triticum aestivum* L.), was used for the culture inoculations, and the F<sub>1</sub> inoculum of all hybrids was obtained from this variety. The

<sup>1</sup> Received for publication June 5, 1942. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Washington Agricultural Experiment Station.

<sup>2</sup> Italic numbers in parentheses refer to Literature Cited, p. 563.

<sup>3</sup> C. I. refers to accession number of the Division of Cereal Crops and Diseases.

winter wheat host testers (7) were used for the pathogenicity tests with hybrid chlamydospores, and the inoculum of segregating generations was selected from one or more of these varieties. The seed of all varieties was rendered smutfree by treating it with formaldehyde solution (1:320) for 5 minutes, after which it was thoroughly washed in running water. All inoculated seed was planted at Pullman, Wash., under environmental conditions presumably favorable for infection.

The smut percentages were determined on the basis of total heads of wheat in a 5- or 6-foot row, the number ranging from 100 to several hundred, depending upon variety, soil, and season. The smut percentages were recorded in whole numbers, fractions of one-half or more being considered whole numbers and those of less than one-half being disregarded.

#### EXPERIMENTAL RESULTS

Results have been obtained from studies on 50 hybrids, 23 of which resulted from crosses between *Tilletia tritici* and *T. levis* and 27 from crosses between races within these species. Of the 23 interspecies hybrids, 19 proved capable of perpetuating themselves while 4 were nonpathogenic on the host varieties used, and of the 27 interracial hybrids, 16 survived several generations of propagation and 11 were nonpathogenic. As yet, no adequate explanation can be offered for the failure of some of these hybrids to survive. In some cases it appeared to be due to failure of the spores to germinate. In other cases, however, germination of the spores was observed but no infection or very slight infection was obtained. Apparently hybrid sterility due to wideness of the crosses is of no great significance in this connection, since there was a greater degree of the nonsurvival quality in the race hybrids than in the species hybrids. Furthermore, certain selfed lines exhibited similar characteristics. Six inbred lines of L-8 and 2 of T-11 could not be propagated beyond the first generation, whereas 6 inbred lines of T-6 and 1 of L-7 were maintained through several generations without any diminution in pathogenicity. Apparently, therefore, there is no correlation between the taxonomic relationship of the parents and the survival quality of hybrids between species and between races of the wheat bunt fungi. It seems significant, however, that the majority of such hybrids can be propagated as readily as their parents and in many instances give rise to new pathogenic types.

#### SPECIES HYBRIDS

The percentages of smut produced by hybrids between L-8 and T-9 are presented in table 1, along with the host reaction to the parent races.<sup>4</sup> Table 1 shows the susceptibility of Oro to race L-8 and its resistance to race T-9, in contrast to the resistance of Hoheneimer to L-8 and its susceptibility to T-9. All of the hybrids between these two races have been tested through the sixth generation, and it appears that at least three types of pathogenicity are represented in the four hybrids listed. Hybrid 35 appeared to be identical in pathogenicity with the L-8 parent, and it also resembled this parent in having smooth spores. Two other hybrids between these two races,

<sup>4</sup> The host reactions to the parent races shown in tables 1 to 3 were obtained over a period of 5 years or longer.



not listed in table 1, were identical with Hybrid 35. Hybrid 38, which had reticulate spores, was unlike either parent in pathogenicity, since it was able to infect only Hybrid 128. On the other hand, Hybrid 39, which also had reticulate spores, appeared to possess a combination of pathogenicity factors from both parents, since it infected both Oro and Hohenheimer. This hybrid is similar to the one described in 1938 (4). Hybrid 40, through the fifth generation, appeared to be distinctly less virulent than either parent on all of the varieties, including the susceptible variety Hybrid 128. In the sixth generation, however, it exhibited a high degree of virulence on Hybrid 128 and Oro, thus resembling Hybrid 35 and the L-8 parent. However, unlike L-8, the chlamydospores of Hybrid 40 were reticulate.

TABLE 1.—Pathogenicity of interspecies hybrids between *Tilletia levis* and *T. tritici*

HYBRID L-8 × T-9								
Parent or hybrid No.	Pedigree	Inoculum		Smut in—				
		Source	Generation	Hybrid 128	Oro	Albit	Hohenheimer	Hussar × Hohenheimer
L-8		Oro		Percent	Percent	Percent	Percent	Percent
T-9		Hohenheimer		90	85		0	
		Hard Federation	F <sub>2</sub>	85	0		30	
		Oro	F <sub>3</sub>	26	12		0	
35	L56-1 × T157-1	do	F <sub>4</sub>	82	70		0	
		do	F <sub>5</sub>	81	84		0	
		do	F <sub>6</sub>	84	77		0	
		do	F <sub>7</sub>	92	85		0	
		Hard Federation	F <sub>2</sub>	48	3		1	
		Oro	F <sub>3</sub>	65	1		0	
38	L56-1 × T157-4	Hybrid 128	F <sub>4</sub>	97	1		0	
		do	F <sub>5</sub>	90	1		0	
		do	F <sub>6</sub>	95	1		0	
		Hard Federation	F <sub>2</sub>	94	33		21	
		Oro	F <sub>3</sub>	85	30		27	
		Hohenheimer	F <sub>4</sub>	90	27		33	
39	L56-1 × T157-5	Oro	F <sub>5</sub>	88	24		22	
		Hohenheimer	F <sub>6</sub>	89	28		32	
		Oro	F <sub>7</sub>	86	28		26	
		Hohenheimer	F <sub>8</sub>	79	30		35	
		Oro	F <sub>9</sub>	87	84		20	
		Hohenheimer	F <sub>10</sub>	96	72		68	
		Hard Federation	F <sub>2</sub>	48	10		0	
		Oro	F <sub>3</sub>	53	1		4	
40	L56-1 × T157-8	Hybrid 128	F <sub>4</sub>	22	7		0	
		do	F <sub>5</sub>	32	9		2	
		do	F <sub>6</sub>	94	64		5	
HYBRID L-7 × T-10								
L-7		Albit		92		93	0	0
T-10		Hohenheimer		93		0	73	0
		Hohenheimer	F <sub>2</sub>	70		0	0	0
41	L70-2 × T67-3	Hybrid 128	F <sub>3</sub>	85			0	0
		do	F <sub>4</sub>	87		1	0	0
		Hindi	F <sub>5</sub>	30		1	0	0
42-1	L70-3 × T67-3	Hybrid 128	F <sub>2</sub>	29		0	1	0
		do	F <sub>3</sub>	98		0	0	0
		Hybrid 128	F <sub>4</sub>	96		95		
42-2	do	Albit	F <sub>5</sub>	92			0	0
		Hybrid 128	F <sub>6</sub>	95		83	5	0
		Hindi	F <sub>7</sub>	56		2	1	0
		Hohenheimer	F <sub>8</sub>	47		1	2	0
44	L71-1 × T69-1	do	F <sub>9</sub>	38			6	0
		do	F <sub>10</sub>	10		1	7	0
		Hindi	F <sub>11</sub>	27		3	1	0
46-1	L71-1 × T69-3	Albit	F <sub>2</sub>	94		88	6	0
		do	F <sub>3</sub>	86			0	0

<sup>1</sup> *T. levis* parent, chlamydospore 56, sporidium 1, crossed with *T. tritici* parent, chlamydospore 157, sporidium 1.

TABLE 1.—Pathogenicity of interspecies hybrids between *Tilletia levis* and *T. tritici*—Continued

## HYBRID L-7 × T-10—Continued

Parent or hybrid No.	Pedigree	Inoculum		Smut in—				
		Source	Generation	Hybrid 128	Oro	Albit	Hohenheimer	Hussar × Hohenheimer
				Percent	Percent	Percent	Percent	Percent
46-2	L71-1 × T69-3	Hohenheimer	F <sub>2</sub>	25	-----	0	5	1
		do.	F <sub>3</sub>	33	-----	3	3	1
		do.	F <sub>4</sub>	6	-----	3	3	0
49	L71-2 × T69-2	Hindi	F <sub>1</sub>	47	-----	3	0	0
		Hybrid 128	F <sub>2</sub>	78	-----	8	4	0
		Hohenheimer	F <sub>3</sub>	37	-----	-----	26	1
		do.	F <sub>4</sub>	7	-----	0	1	0

## HYBRID T-12 × L-8

T-12	-----	Hohenheimer	-----	92	1	-----	67	-----
L-8	-----	Oro	-----	90	85	-----	0	-----
		Hindi	F <sub>1</sub>	75	7	-----	7	-----
		Oro	F <sub>2</sub>	86	36	-----	6	-----
67	T192-1 × L74-1	do.	F <sub>3</sub>	88	82	-----	15	-----
		Hohenheimer	F <sub>2</sub>	81	16	-----	61	-----
		do.	F <sub>3</sub>	77	14	-----	74	-----
68	T192-1 × L74-2	Hindi	F <sub>1</sub>	30	2	-----	0	-----
		Hybrid 128	F <sub>2</sub>	66	1	-----	11	-----
		do.	F <sub>3</sub>	19	1	-----	1	-----
		Hindi	F <sub>1</sub>	63	5	-----	18	-----
73	T192-1 × L74-3	Hohenheimer	F <sub>2</sub>	81	12	-----	29	-----
		do.	F <sub>3</sub>	63	1	-----	27	-----

The relative pathogenicity of hybrids between races L-7 and T-10 is likewise presented in table 1. Albit and Hussar were susceptible to L-7 and resistant to T-10, whereas Hohenheimer was resistant to L-7 and susceptible to T-10. (The reaction of Hussar to these two races is not shown in table 1.) The variety Hussar × Hohenheimer (C. I. 10068-1) was highly resistant to both of these races as well as to all other known races of the wheat bunt fungi. This variety was selected from a cross between Hussar (C. I. 4843) and Hohenheimer (C. I. 11458). The primary purpose, therefore, in hybridizing L-7, which infects Hussar, and T-10, which infects Hohenheimer, was to test the theoretical possibility of obtaining segregates capable of infecting Hussar × Hohenheimer. It is apparent, however, from the data in table 1, that such segregates did not occur, since this variety was highly resistant to all the hybrids. Furthermore, most of the hybrids were less virulent than either of the parent races. For example, Hybrids 41 and 42-1 infected only the susceptible variety Hybrid 128. At least one other hybrid not included in table 1 gave a similar reaction. Hybrids 42-2 and 46-1 produced the same reaction as the L-7 parent, although, like the T-10 parent, the chlamydospores of both of the selections were reticulate. The remainder of the hybrids between L-7 and T-10 shown in table 1 infected Hybrid 128 only (except Hybrid 49, which produced 26 percent on Hohenheimer in the F<sub>3</sub>), and this capacity appeared to decline in successive generations, especially when the inoculum was taken from Hohenheimer.

Data on the pathogenicity of hybrids between T-12 and L-8 are also presented in table 1. T-12 infects Hohenheimer but not Oro, whereas L-8 infects Oro but not Hohenheimer. Of the three hybrids studied, Hybrid 67 was the most virulent, being able to infect both Oro and Hohenheimer, whereas Hybrid 68 infected only Hybrid 128. Although it infected Hohenheimer, Hybrid 73 was less virulent on this variety than the T-12 parent. The chlamydospores of Hybrid 68 were smooth while those of the other two hybrids were reticulate.

## RACE HYBRIDS

The data obtained from studies on hybrids between T-8 and T-9 are presented in table 2. Albit and Hussar are susceptible to T-8 and resistant to T-9, whereas Hohenheimer is resistant to T-8 and susceptible to T-9. As noted previously, Hussar  $\times$  Hohenheimer is resistant to these and all other known races of *Tilletia tritici* and *T. levis*. It will be noted, however, that all the hybrids between T-8 and T-9 included in table 2, except Hybrid 53, were capable of infecting this variety. Three other hybrids between these races, not included in table 2, were similar to Hybrid 53 in their pathogenicity. In general, pathogenicity on Hussar  $\times$  Hohenheimer was accompanied by greater virulence on Hohenheimer than that exhibited by the T-9 parent, and by less pathogenicity on Albit and Hussar than that exhibited by the T-8 parent. An exception to this may be noted in the case of Hybrid 56, which was more virulent on Hohenheimer and Albit than the parent races, though less virulent on Hussar than T-8. A preliminary report on the pathogenicity of hybrids between T-8 and T-9 and the transgressive inheritance of pathogenicity factors in some of these hybrids has been already made (6).

TABLE 2.—Pathogenicity of interracial hybrids of *Tilletia tritici*HYBRID T-8  $\times$  T-9

Parent or hybrid No.	Pedigree	Inoculum		Smut in—				
		Source	Genera- tion	Hybrid 128	Albit	Hohen- heimer	Hussar $\times$ Hohen- heimer	Hussar
				Percent	Percent	Percent	Percent	Percent
T-8		Albit		85	89	0	0	53
T-9		Hohenheimer		85	0	30	0	0
		Hindi	F <sub>1</sub>	95	12	28	3	0
		Hussar $\times$ Hohenheimer	F <sub>2</sub>	86		37	8	
		do	F <sub>3</sub>	75		35	16	
		do	F <sub>4</sub>	86	19	74	29	2
50	T 60-1 $\times$ T 157-2	Hohenheimer	F <sub>2</sub>	99	20	37	4	
		do	F <sub>3</sub>	86		31	1	
		do	F <sub>4</sub>	88	13	62	2	0
		Hybrid 128	F <sub>2</sub>	94	13	0	0	
		Albit	F <sub>2</sub>	94	83	0	0	
		Hindi	F <sub>1</sub>	94	8	10	3	
52	T 60-1 $\times$ T 157-4	Hussar $\times$ Hohenheimer	F <sub>2</sub>	90		27	6	
		do	F <sub>3</sub>	90		50	18	
		do	F <sub>4</sub>	87	71	77	36	27
		Hindi	F <sub>1</sub>	72	3	1	0	
53	T 60-1 $\times$ T 157-5	Hybrid 128	F <sub>2</sub>	63	1	0	0	
		do	F <sub>3</sub>	74		1	0	
		do	F <sub>4</sub>	23	1	1	0	0

<sup>1</sup> T-8 parent, chlamydospore 60, sporidium 1, crossed with T-9 parent, chlamydospore 157, sporidium 2.

TABLE 2.—Pathogenicity of interracial hybrids of *Tilletia tritici*—Continued

## HYBRID T-8 × T-9—Continued

Parent or hybrid No.	Pedigree	Inoculum		Smut in—				
		Source	Generation	Hybrid 128	Albit	Hohenheimer	Hussar × Hohenheimer	Hussar
				Percent	Percent	Percent	Percent	Percent
54	T60-2×T157-1	Hindi	F <sub>1</sub>	86	16	18	1	
		Hohenheimer	F <sub>2</sub>	86	5	75	12	
		Hussar × Hohenheimer	F <sub>3</sub>	95		29	13	
		do	F <sub>4</sub>	92	20	67	37	3
55	T60-2×T157-2	Hindi	F <sub>1</sub>	93	9	20	5	
		Hussar × Hohenheimer	F <sub>2</sub>	92		36	19	
		do	F <sub>3</sub>	89	30	85	56	7
		Hohenheimer	F <sub>4</sub>	99	81	74	25	
56	T60-2×T157-3	do	F <sub>1</sub>	85		54	4	
		do	F <sub>2</sub>	88	81	84	5	17
		Hussar × Hohenheimer	F <sub>3</sub>	93	12	24	2	
		Albit	F <sub>4</sub>	85		38	7	
		do	F <sub>1</sub>	99	82	13	9	
		do	F <sub>2</sub>	94		2	8	
		do	F <sub>3</sub>	94		89	44	
		Hussar × Hohenheimer	F <sub>4</sub>		85			29

## HYBRID T-8 × T-10

T-8		Albit		85	89	0	0	93
T-10		Hohenheimer		93	0	73	0	0
61	T61-1×T67-1	Hindi	F <sub>1</sub>	33			0	
		Hybrid 128	F <sub>2</sub>	92	0	0	0	
		do	F <sub>3</sub>	93		0	0	
		do	F <sub>4</sub>	75	0	0	0	0
62	T61-1×T67-2	Hindi	F <sub>1</sub>	91			3	
		Hussar × Hohenheimer	F <sub>2</sub>	92		64	18	
		do	F <sub>3</sub>	88		67	57	
		do	F <sub>4</sub>	84	63	92	35	22
63	T61-1×T67-3	Hindi	F <sub>1</sub>	83			0	
		Hybrid 128	F <sub>2</sub>	30	0	0	0	
		do	F <sub>3</sub>	78		0	0	
		do	F <sub>4</sub>	95	4	0	0	0

Hybrids were also made between T-8 and T-10 to determine whether Hussar × Hohenheimer might be susceptible to any of the segregates. These races differ in the same manner as that described for T-8 and T-9. However, T-10 differs from T-9 by its greater virulence on Hohenheimer (?). The relative virulence of three hybrids between T-8 and T-10 is indicated by the data presented in table 2. Hybrids 61 and 63 were able to infect only Hybrid 128, whereas Hybrid 62 infected all of the varieties including Hussar × Hohenheimer, though it was less virulent on Hussar than the T-8 parent.

## BACKCROSSES

Four backcrosses to the T-8 parent were made with two monosporidial lines from each of two chlamydospores from Hybrid 50 (T-8 × T-9). The virulence of these backcrosses is shown by the data presented in table 3, and it will be noted that Albit was highly susceptible to all of them as well as to the T-8 parent, whereas Hohenheimer exhibited varying degrees of susceptibility, depending upon the source of the inoculum (table 3).



TABLE 3.—Pathogenicity of backcrosses of Hybrid 50 to the T-8 parent

Parent and backcross No.	Pedigree	Inoculum		Smut in—		
		Source	Generation	Hybrid 128	Albit	Hohenheimer
T-8		Albit		Percent	Percent	Percent
		Hindi		85	89	0
74	T60-1×H91-3 <sup>1</sup>	Albit	F <sub>1</sub>	63	53	4
		Hybrid 128	F <sub>2</sub>	76		21
		Hindi	F <sub>3</sub>	85	84	6
		Hohenheimer	F <sub>1</sub>	90	75	31
75	T60-1×H91-7	do	F <sub>2</sub>	92		84
		Albit	F <sub>2</sub>	87	80	82
		Hybrid 128	F <sub>3</sub>	88		55
		Hindi	F <sub>3</sub>	94	93	17
		Albit	F <sub>1</sub>	95	76	32
76	T60-1×H92-1	Hybrid 128	F <sub>2</sub>	85		19
		Hohenheimer	F <sub>3</sub>	93	97	9
		do	F <sub>2</sub>	91		80
		Hindi	F <sub>3</sub>	96	91	84
		Albit	F <sub>1</sub>	76	81	14
78	T60-1×H92-5	Hybrid 128	F <sub>2</sub>	83		19
		Hohenheimer	F <sub>3</sub>	91	93	40
		do	F <sub>2</sub>	86		60
			F <sub>3</sub>	94	79	73

<sup>1</sup> Chlamydo-spore 60, sporidium 1, of T-8, crossed with chlamydo-spore 91, sporidium 3, of Hybrid 50.

## DISCUSSION

Hybridization has long been recognized as a possible factor in the origin of physiologic races of *Tilletia tritici* and *T. levis*. Although these species and their respective races can be hybridized readily under controlled conditions, the extent to which this process occurs under natural conditions is not known. Nevertheless it seems highly significant that many of the artificially produced hybrids have given rise to one or more new pathogenic types. In other words, the readiness with which this process functions under controlled conditions suggests that it probably occurs in nature at least occasionally. Furthermore, the results obtained from these studies tend to emphasize the possible importance of hybridization as a factor in perpetuating the bunt problem.

The importance of genetic factors in the basic inheritance of pathogenicity in the bunt fungi is evident from the data presented in the foregoing pages. However, the exact nature of this inheritance has not been determined, mainly because of the difficult, if not impossible task, of obtaining cultures of complete sets of primary sporidia from parent and hybrid chlamydo-spores.<sup>5</sup> Nevertheless, there appears to be sufficient evidence at hand to indicate that in hybrids involving differential reaction on two host varieties pathogenicity is inherited on at least a dihybrid basis. For example, at least three pathogenic types are represented by the four hybrids between L-8 and T-9 (table 1). Four pathogenic types would be expected where two factors are involved. These hybrids were produced by crossing one monosporidial line of L-8 with four monosporidial lines of T-9; two of the hybrids

<sup>5</sup> Hanna (2) perfected a technique which made it possible to obtain cultures of all the sporidia from a promycelium, but the method was not described.

are entirely different from both parents, whereas the other two appear to be the same as the L-8 parent. Therefore, at least three factor combinations appear to have occurred in these four hybrids. Similar results were obtained with hybrids between T-12 and L-8 (table 1).

Evidence of multiple factors and the extent to which new combinations of pathogenicity factors may occur in hybrids of the bunt fungi is indicated by results obtained with hybrids between T-8 and T-9 and T-8 and T-10 (table 2). Several of these hybrids produced segregates that were pathogenic on Hussar  $\times$  Hohenheimer, a variety that is highly resistant to all known races of *Tilletia tritici* and *T. levis*. Apparently the dominant factor in the production of pathogenicity for Hussar  $\times$  Hohenheimer was carried by certain sporidia of the T-9 parent. This is indicated by the pathogenic properties exhibited by Hybrids 50, 52, and 53. In pedigree, these hybrids differ only by the T-9 parent, and the fact that Hybrid 53 was nonpathogenic on Hussar  $\times$  Hohenheimer suggests that sporidium 5 of the T-9 parent lacked the factor or factors necessary to produce pathogenicity on this variety. Similarly the results obtained with Hybrids 50 and 55 suggest a dominant influence of the T-8 parent on the reaction of Albit. The pedigree of these hybrids differs on the side of the T-8 parent, and segregates of Hybrid 55 that were highly virulent on Hohenheimer and Hussar  $\times$  Hohenheimer were more virulent on Albit than similar segregates of Hybrid 50. In other words, the factor or factors for virulence on Albit in sporidium 1 of the T-8 parent were different from those in sporidium 2. Furthermore, the greater susceptibility of Hohenheimer to certain segregates of Hybrids 50 and 55 than to the T-9 parent suggests that the T-8 parent contributed to the virulence of these segregates on this variety.

The high virulence on Hohenheimer exhibited by Hybrid 50 was evident also in backcrosses of this hybrid to the T-8 parent (table 3). Three of the backcrosses were more virulent on Hohenheimer than the T-9 parent, and they were equally as virulent on Albit as the T-8 parent. Furthermore, it seems probable that the other backcross (Hybrid 74) would also have been highly virulent on Hohenheimer, if the inoculum had been taken from that variety.

Although pathogenicity in *Tilletia tritici* and *T. levis* apparently is controlled by genetic factors, the selective influence of the host variety plays an important part in the expression of pathogenic properties and hence in the establishment of new physiologic races. Evidence of this is shown by the results obtained with several of the hybrids used in these studies. For example, the repeated selection of inoculum of Hybrid 39 (table 1) from Oro through the sixth generation resulted in a marked increase in virulence on Oro with no change in virulence on Hohenheimer, whereas the selection of the inoculum of this hybrid from Hohenheimer resulted in an increase in virulence on both Oro and Hohenheimer. In contrast, a marked decrease in virulence was exhibited by Hybrids 46 and 49 (table 1), even on the susceptible variety Hybrid 128, when the inoculum was repeatedly taken from Hohenheimer. The influence of host selection on pathogenicity is further indicated by the infection of Hussar  $\times$  Hohenheimer by hybrids between T-8 and T-9 (table 2). The inoculum that was taken repeatedly from Hussar  $\times$  Hohenheimer increased in virulence on this variety, whereas selections of the same hybrids taken from

other varieties decreased in virulence on Hussar  $\times$  Hohenheimer. Similar results were obtained with other hybrids (table 2).

Independent inheritance of factors for pathogenicity, and for spore morphology, is indicated by the results obtained with Hybrids 42-2 and 46-1 (table 1). Both of these hybrid segregates possessed the pathogenicity of the L-7 parent and the spore morphology of the T-10 parent.

#### SUMMARY

The results from investigations on 50 interspecies and interracial hybrids of *Tilletia tritici* and *T. levis* are presented. Approximately 83 percent of the interspecies hybrids perpetuated themselves, in contrast to only 59 percent of the interracial hybrids. On the other hand, the race hybrids were more productive of new pathogenic segregates than the species hybrids.

Some of the segregates from the various hybrids were more virulent than the parent races, others were less virulent, while still others were similar in pathogenicity to the parent races.

Entirely new combinations of pathogenicity factors were produced in several of the hybrids, as indicated by their pathogenicity on the variety Hussar  $\times$  Hohenheimer, which is highly resistant to all known naturally occurring races of the wheat bunt fungi.

Pathogenicity in *Tilletia tritici* and *T. levis* is genetically controlled and apparently inherited on a multiple-factor basis. Factors for pathogenicity and spore morphology are inherited independently.

The selective influence of the host variety is important in the establishment of new pathogenic types resulting from hybridization.

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